

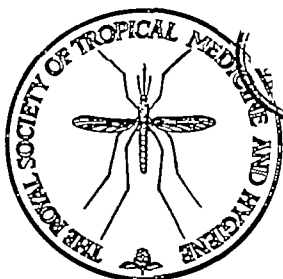


TRANSACTIONS

OF THE

ROYAL SOCIETY OF TROPICAL  
MEDICINE AND HYGIENE

PATRON - HIS MAJESTY THE KING



ZONAE TORRIDAE TUTAMEN

VOL. XXXIX 1945 1946

London

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE,  
MANSON HOUSE, 25 PORTLAND PLACE, LONDON W1

Telephone: LANSHAM 2127

Telegrams: ANOPHELES, LONDON



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# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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VOL. XXXIX. No 1 SEPTEMBER, 1945

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## LABORATORY MEETING

of the Society held at

The Royal Army Medical College, Millbank, London,

on

Thursday, 15th March, 1945, at 3 p.m.

Major General A G BIGGAM, C B K.H.P. M.D. F.R.C.P. (late R.A.M.C.)  
Vice-President, in the Chair

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## DEMONSTRATIONS

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The Royal Army Medical College

### A Personal protection of the soldier against malaria

Model of soldier wearing jungle clothing

Nylon hammock, pitched with protective mosquito net.

Suppressive mepacrine, 0.1 gramme tablets —

(a) In 7-day cellophane strips.

(b) In tins of seven.

(c) In tins of 1 000

A Mepacrine Parade

Dimethyl phthalate repellent in container, as issued.

Wallet containing head net, sleeves and oversocks, the whole impregnated with D.M.P.

Individual Sparklet.

Insecticide (Freon) bomb

### B Prevention of typhus fever

#### (i) Louse-borne typhus

Model dressed in protective clothing for handling cases of typhus.

• Chart showing lousiness in last war and in this.

DDT impregnated shirt.

Dust guns hand and power-operated.

A.L. 63 (Mark III) powder containing DDT

Typhus vaccine.

(ii) *Scrub Typhus* (in conjunction with Major K. Mellanby)

Method of impregnating clothing with dibutyl phthalate

The vector and its habitat.

How to get scrub typhus "

C. Ration packs for Pacific theatre

Emergency ration.

24 hour ration for one man.

Composite six man ration for 1 day (Types 1 and 4)

D. Purification of water in forward areas.

The midget metafilter for small units.

Millbank individual filter bag

Individual water sterilizing outfit, latest type, double ended.

Soldier's water bottle new type.

E. Prevention of tropical sores

Individual first aid outfit.

F. Estimation of mepacrine in blood

Photo-electric fluorimeter

G. 1. The experimental effects of penicillin on cultures of *L. donovani* in Adler's medium and *E. histolytica* in Dobell's HS/hs medium were outlined with the estimated inhibitory and lethal concentrations. Graphs were shown of an "enhancement effect" on the growth of *Entamoeba histolytica* by certain sub-lethal concentrations of penicillin.

2. Microscopical preparations were shown of thick and thin films of *P. croax* and *T. brucei* stained with J.S.B. stain to show the sharp differential staining and general utility of this stain for demonstrating parasites in thick and thin films. (Ref. *Ind. med. Gaz.* 1944 79 102).

Lt.-Col. J. Bryant.

Photographs of mosquito breeding places and control measures against anophelines in Palestine

Dr E. Burt and Dr H. Fairbairn (shown by Dr C. A. Hoare)

Preparations of *Trypanosoma rhodesiense*

(1) *Metacyclic trypanosomes* ejected by the tsetse-flies in the act of biting

Preparations of the salivary forms (metacyclic trypanosomes, etc.) were obtained by the following method —

(a) A microscopic slide is smeared with a thin film of egg albumen and allowed to dry

(b) An infected tsetse fly is placed in a bottle, the mouth of which is covered with mosquito gauze

(c) The bottle is held in a rack, mouth downwards, and a gumcap is placed under it.

(d) As the hungry tsetse-fly extrudes its proboscis through the gauze in an attempt to feed, the prepared slide is interposed between the fly and the gumexig with the result that the fly probes on the slide ejecting some of the saliva containing the trypanosomes

(e) The salivary smear is then dried in the air and stained like a blood-film by one of Romanovsky's methods.

#### (II) Trypanosomes in the primary nodule

In the great majority of volunteers infected through the medium of a tsetse fly there develops a local reaction at the site of the bite. This reaction starts as a nodule under the skin. It is at first about the size of a pea, but it gradually increases in dimensions until it becomes an indurated swelling measuring about 3 x 2 inches. It is covered with minute vesicles at the summit, and is tense and tender.

Withdrawal of some of the fluid by puncture of the nodule reveals the presence of trypanosomes some days before they invade the blood stream.

Since bites by non infected tsetse do not produce this reaction, the nodule (or trypanosome chancre) represents the primary lesion, and is therefore the earliest diagnostic character of human trypanosomiasis (both in the *rhodesiense* and *gambiense* forms). As a rule however it escapes detection because patients are rarely seen in the early stages of the infection.

Dr J R Busvine and Mrs S Barnes, Ph D

#### DDT impregnated materials testing methods etc

An impregnated louse proof shirt was shown on which the DDT was imperceptible. Small blocks of materials used in building (painted and unpainted wood, plaster glass etc.) bearing insecticidal films of DDT were exhibited. The films of crystals were quite visible on dark or impervious surfaces even a year after application. The bug killing powers of these films are tested at intervals and the method (by confining bugs on the treated surfaces) was demonstrated.

Bed bugs and body lice exhibiting symptoms of DDT poisoning were shown.

Dr E H Derrick (shown by Dr C M Wenyon)

#### An unusual case of amoebiasis. Invasion of the tissues by an amoeba which is not *Entamoeba histolytica*.

A Japanese prisoner was admitted to hospital in Brisbane on 4.3.43. He was treated for malaria and dysentery but died on 9.3.43. At postmortem (17 hours after death) the stomach, small intestine and large intestine were ulcerated. The lungs were consolidated while the brain contained a haemorrhagic mass (2 x 1 x 1½ inches) on the lateral aspect of the right parietal lobe. Sections of the organs revealed large numbers of amoebae in all the intestinal ulcers, the mesenteric glands, the lungs and the brain. These amoebae are evidently not *Entamoeba histolytica* nor *E. coli*. Of the known human amoebae

they resemble most closely *Iodamoeba bütschlii* which, however is regarded as a harmless inhabitant of the intestine. It has never been known to invade the tissues. In this case however it has to be assumed that this is the first time that such an invasion has been noted and that *I. bütschlii* has carried out an extensive penetration of the tissues, or which seems less likely that an entirely new human amoeba is involved. The full details of the case are to be published elsewhere.

Prof H J Dible Dr S Sherlock and Royal Army Medical College

Demonstration of sections of liver obtained by liver puncture biopsy from patients who had taken suppressive meprobamate therapy from 6 to 17 months. These livers appeared healthy and showed only slight physiological variations from normal.

Prof R M Gordon Prof T H Davey and Mr K Unsworth

The control of scabies by the use of Tetmosol soap.

1 Sections of skin, cut on a freezing microtome, of rats infected with a mite (*Notodres*) causing scabies. The animals were treated with tetraethylthiuram monosulphide ("tetmosol") in a coconut oil base and the skin removed at various intervals after rubbing in the drug. The sections show that the drug penetrates into the hair follicles within 15 minutes of its application and that it reaches all stages of the mites in the burrows, but that 24 to 48 hours are required before all the mites and, particularly their ova are destroyed.

2. Colour photographs of the skin of rats which had been exposed to infection with *Notodres* from their fellows. The photographs show that, whereas ordinary soap and soap impregnated with benzyl benzoate have no protective action against the acquirement of the disease, soap impregnated to the extent of 5 per cent. with tetraethylthiuram monosulphide, when used once daily renders the animals immune.

3 A graph illustrating the rise in the incidence of scabies in a closed human community of 400 persons when using ordinary soap and its subsequent fall following the substitution of 5 per cent. tetmosol soap.

Lt-Col W H Hargreaves

Radiological appearances of calcified cysts of *Toxocara solium* in brain.

It has been found that high penetration is effective in showing up calcified cerebral cysticerci in detail. The calcification commonly appears in the scolex only but quite frequently a calcified cyst wall may be seen like a shell or halo outside the scolex, and occasionally calcification can be seen starting in the cyst wall first.

Eight radiographs were demonstrated, and one particularly striking film was shown stereoscopically

Dr F Hawking

A Growth of parasites in tissue culture

(i) Exoerythrocytic forms of *Plasmodium gallinaceum*

Tissue cultures were demonstrated which had been prepared from the

spleens of chickens infected 8 days previously with sporozoites of *P. gallinaceum*. The macrophages in these cultures contain exoerythrocytic forms of *P. gallinaceum* which undergo a regular cycle of development in the cells. A fuller description of these cultures was given in these TRANSACTIONS in August, 1944. In recent experiments large numbers of sporozoites have been injected intravenously into a small chick. Three hours later the chick was killed and tissue cultures were made from the spleen. After about 10 days typical exoerythrocytic forms were found in the cells of the culture.

(ii) *Trypanosoma cruzi*

*T. cruzi* has been grown in tissue culture by the same technique as used for *P. gallinaceum*. The tissue consists of embryonic rat heart grown in a medium containing rat serum, etc. After 3 days blood from mice infected with *T. cruzi* was inserted. The trypanosomes enter the cells and undergo a cycle of multiplication which lasts about 5 days. At the end of this time new trypanosomes emerge from the cells and are available to infect clean cells. Cultures have been maintained by this technique for 59 days.

(iii) *Leishmania donovani*

The parasites were cultivated by the same technique as that used for *P. gallinaceum*.

Infected hamsters were kindly supplied by Mr L. G. GOODWIN. Tissue cultures were made from the spleen in a medium containing 20 per cent. hamster serum. They were incubated at 37° C. but during handling the cultures were allowed to cool to room temperature for about 3 hours every 3 days. Growth of the cells was fair; the cultures were maintained for 23 days, after which they became infected with bacteria. After 20 days large numbers of free flagellates appeared in the fluid medium.

B *Filaria* from the cotton-rat *Leptomyscus carini*.

Experimental studies of filariasis have been greatly handicapped in the past by lack of a suitable experimental animal. Recently it has been found that cotton rats from the southern states of America are often infected with a worm known as *Leptomyscus carini*.

A batch of wild cotton rats was recently received by Dr C. H. ANDREWES from America and it was found that about half the animals were infected with these worms. Specimens were demonstrated which had been obtained from these rats. The adults live in the pleural space and microfilariae occur in the peripheral blood. The microfilariae are non-periodic and sheathed. The insect vector is not yet known.

These parasites offer great promise for chemotherapeutic experiments (See J. T. CULBERTSON and H. M. ROSE, 1944 *J. Pharmacol. & exp. Therap.*, 81, 189).

A. D. Loes, Ph.D

Living *Ornithodoros delenceni* active

This is the largest known species of *Ornithodoros*. The female ticks

exhibited are the type specimens of this subspecies (Whittrick, 1938) and were discovered buried in sand in a cave in British Somaliland. The natural host is unknown.

This species has exceptional powers of resisting desiccation and starvation.

Major H. S. Leeson (shown by Professor Buxton)

Recent photographs anti-malarial work in Palestine.

Dr K. Mellanby (shown by Professor Buxton)

Larvae and adults of *Trombicula deliensis* from Ceylon (Mounted in lacte phenol polyvinyl alcohol)

The material had been brought to England alive by air and a film record made of the living mites. The identification is tentative.

Air Commodore T. C. Morton Microscopic specimens.

A. A case of rhinosporidiosis in an Indian male aged 23.

*History*—Removal of polyp from right nostril five times during the last 5 years. Already diagnosed as *Rhinosporidium* in India. (Patient comes from North India.)

*Examination*—Pinkish polyp attached to free margin of the right inferior turbinate. The rest of the nasal passage on this side and the left side are healthy. X ray Sinuses—negative.

17th August 1942—Operation—removal of mass.

March 1943—Recurrence of growth, which this time has spread slightly and of which some was attached to the septum. Growth removed and base diathermized.

May 1943—No further recurrence but there is a small area on medial side of right inferior turbinate which bleeds easily. It was from this area that the mass was growing. This area was therefore diathermized.

July 1943—Nose healthy. Accepted as fit for commission.

March 1945—As far as is known has had no further recurrence.

B. *Schistosoma haematobium* and carcinoma of the bladder

Two sections are shown, both obtained from biopsy through a cystoscope from French officers who had served in Syria and in the Lake Chad region of Equatorial Africa. They were both in the early thirties. In one case a definite carcinoma of the bladder is present, together with scanty *Schistosoma haematobium* eggs. In the second, eggs are numerous, together with solid columns of epithelial cells, although palisading and hyperchromatism are not present the epithelial hyperplasia is so marked and metaplasia is present in one area that the condition, though not definitely malignant, is certainly precancerous.

C. Lymph gland showing *Leishmania donovani*.

This lymph gland was obtained by biopsy from a case of kala-azar contracted in Malta. *Leishmania* were also found in the sternal marrow and the spleen was enlarged. The *leishmania* are present in such numbers that a gland puncture would certainly have been found positive.

This case was recorded in the *British Medical Journal*, 1943 by Group Captain F. E. LIPSON and S/Ldr GIBSON.

#### D Blastomycosis

This patient, a healthy young R.A.F. officer of 25 had served 5 years in the Middle East, including Iraq. He spent 1 day in Madagascar and at the Cape. A month after his return to the United Kingdom a raised dusky papular area appeared over his elbow. This after some weeks slowly increased in size and some discharge escaped from minute openings in the epidermis when the skin was placed on the stretch, but there was no pain or irritation. Blastomycosis was suspected, and on applying pressure to the lesion small beads of pus escaped from minute openings in the epidermis. The Leishman stained smears showed scanty thick walled spherical spheres which resembled *Coccidioides immitis*. Cultures unfortunately were overgrown with staphylococci. Treatment with potassium iodide, 90 grains a day was recommended and arrangements made for X-ray treatment. He was seen after treatment and the lesion appeared much drier only a little pus could be expressed from the pits and no fungi could be found in direct smears and attempts at culture, both aerobic and anaerobic, were unsuccessful. Material was sent to Dr J. T. DUNCAN at the E.M.S. Laboratory at Winchester and again cultures were unsuccessful. Local treatment with penicillin was tried without success, and finally a surgical excision was carried out followed by a skin graft. This was successful.

Mr P. G. Shute

Malaria parasites in thick films stained by a modified Field's stain.

#### *Method of using Field's stain*

Between immersion in the azur I methylene blue and the eosin solutions, films are bathed in the isotonic phosphate buffer solution for 1 to 3 seconds instead of in distilled water as suggested by FIELD. This removes most of the haemoglobin without distorting the leucocytes.

Intensification of staining can be obtained by restaining with very weak Leishman. After the film is dry following staining with Field's one drop of Leishman is allowed to act for 2 or 3 seconds and is then diluted with eight to ten drops of distilled water and stained for a further 5 to 10 minutes. By this double method Schüffner's dots stain prominently, even in very young ring forms. Counter staining with Giemsa's stain is less satisfactory than Leishman's.

#### *Very thick films*

When films which are very thick (thick drops) are sent to the laboratory FIELD's rapid method is unsatisfactory. Such films should be soaked in a jar of phosphate buffer solution until most, but not all, of the haemoglobin has been dissolved out. The films are then passed through the stains in the ordinary way.



*Old thick films*

Films may be stained satisfactorily up to at least 28 days after their preparation if before being stained, they are soaked in the phosphate buffer solution until the haemoglobin begins to dissolve out. This may take up to half an hour.

*Films adhering to the slide*

Thick films which are stained as soon as they have dried tend to peel off during the process of staining. This can be prevented by passing the films through the flame of a spirit lamp five or six times before staining. Films which are not stained for 24 hours adhere firmly.

At the present time, when the microscopical diagnosis of malaria is being carried out on a very large scale mostly by workers who are neither malariologists nor protozoologists, and the thick film method of diagnosis is in general use, any extra time spent in staining which will enable parasites and the species to which they belong to be diagnosed with accuracy is time well spent. It saves much time during the actual examination of the film both as regards whether an organism is a malaria parasite or not and it also makes species diagnosis easy. Counter staining thick films with weak Leshman's stain following Field's staining is well worth the little extra time involved.

*Mr H J Sutton**Polyvinyl alcohol: a clearing and mounting medium*

Polyvinyl alcohol (PVA) is one of the plastics and is obtained as a white powder. There are various grades of powder but only one of them is suitable for the medium. This is grade RH.349 A.

The powder when mixed with water—it is not soluble in any of the ordinary fat solvents—forms a viscous solution of a syrupy consistency. This solution when dry leaves a tough transparent film which is resistant to water (unless soaked for several hours), alcohol, ether, xylol, acetone and other solvents.

The solution can be used for covering stained blood films before an immersion oil has been applied, for although it slightly affects the stain when first run on the slide it soon dries hard and can then be cleaned without any danger to the smear.

It is also useful for the examination of living insects, including those which live in water. A drop of the solution is placed on a slide and the specimen immersed in it: this immobilizes the specimen and it can be examined for upwards of half an hour when it can be soaked off in fresh water where it soon recovers.

If the PVA solution is mixed with phenol and lactic acid it kills, clears and mounts the specimens and does not require ringing although it is advisable to use an excess of the medium to allow for shrinkage.

The refractive index is the same as Canada balsam.

Mr J M Watson

The identity of Schaudinn's *Balantidium minutum*.

In 1899 SCHAUDINN described what he believed to be a new species of intestinal ciliate of man to which he gave the name *Balantidium minutum*.

The ciliate was obtained from the stools of a patient suffering among other things from diarrhoea but SCHAUDINN was of the opinion that it was a non pathogenic form.

This organism has only been reported on a few subsequent occasions, despite the many thousands of faecal examinations that are made annually and for this reason BRUMPT, in 1922, suggested that it was probably coprozoic, i.e., a free living species which had accidentally been introduced into the voided faeces and had multiplied in this habitat.

In 1926 WIGHT reported that the free living ciliate *Balantiophorus minutus* Schewiakoff which occurred occasionally in the water-supplies of the Palo Alto district of California, had from time to time been found in human stools in that locality and had been recorded as *Balantidium minutum*.

In 1934 a sample of human stool was sent to these laboratories which contained this coprozoic ciliate *Balantiophorus minutus*.

A detailed study of the structure and relationships of *Balantiophorus minutus* was made, and after a careful and detailed comparison with SCHAUDINN's original description of *Balantidium minutum*, it was concluded that the two species were identical and that SCHAUDINN had actually been dealing with SCHEWIAKOFF's ciliate present in his faecal specimens as a contaminant.

A full account of this work will appear shortly.

Living specimens in human faeces and stained preparations were exhibited.

Dr V B Wigglesworth

The fate of haemoglobin in *Rhodnius prolixus* (Hemiptera)

In *Rhodnius* most of the haemoglobin ingested is broken down in the lumen of the gut to protohaematin which is excreted unchanged. But a small amount is absorbed and circulates in the haemolymph as kathaemoglobin (parahaematin). This is taken up by the salivary glands where it appears as a cherry red pigment with properties similar to haemalbumin. Blood pigment is also transferred to the yolk of the eggs and becomes concentrated in the stomach of the newly hatched nymph as a bright red fluid (parahaematin). In the next few days most of this is digested in the gut to give protohaematin some is transferred to the salivary glands to give rise to their usual pigment.

Blood pigment in the haemolymph of *Rhodnius* is taken up also by the pericardial nephrocytes and by the epithelial cells of the stomach and intestine. Here it is converted to a brown pigment (a modified haem pigment), to a green pigment (probably of the verdohaem type and resembling choleglobin) and finally to biliverdin. Biliverdin accumulates throughout life in the pericardial cells which become bright green. In the gut it is discharged to the lumen and

appears in the faeces. The free iron accumulates throughout life in the cells of the stomach and intestine in old insects there are heavily laden with iron deposits.

After the injection of haemoglobin into the haemolymph all the above processes are exaggerated. In addition, some breakdown of blood pigment takes place in the Malpighian tubes and the lumen of the tubes may become charged with massed droplets of biliverdin displaced from the pericardial cells.

Dr E. J. King and Mrs. Glehrst, Ph.D.

*Field methods for estimating mepacrine in urine, blood and plasma.*

A modification of the MASEN (1943) technique has been worked out to enable the estimation of mepacrine in plasma or whole blood to be performed under field conditions.

*Special apparatus required*—Portable visual fluorimeter consisting of a boxed in mercury light, with a trench or holes for test tubes screened by Wood's glass, which lets through the ultra-violet and no visible light.

100 ml. separating funnel.

Non-fluorescent test tubes (e.g. Chance's bysil,  $\frac{1}{2} \times 6$  inches).

Centrifuge, small electric or hand.

*Reagents*—10 per cent. NaOH.

0.3 N-NaOH.

Petroleum ether

Redistilled isopropyl alcohol and redistilled isobutyl alcohol mixed in equal proportions.

30 per cent. of isopropyl alcohol in 0.1 N-HCl.

Standard mepacrine solution (one mepacrine pill (0.1 gramme) shaken up in 1 litre 0.1 N-HCl).

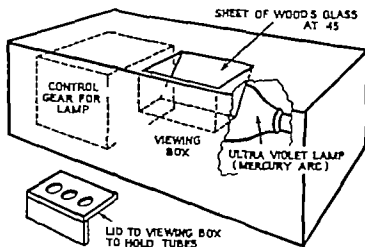
Borate NaOH buffer (4 gramme sodium borate in 100 ml. 1.35 N-NaOH, filtered).

*Method*—In a separating funnel are placed 2 ml. 10 per cent. NaOH, 25 ml. petroleum ether and 25 ml. of the isopropyl-isobutyl alcohol mixture. 10 ml. plasma, or 5 ml. whole blood + 5 ml. water are added and the mixture shaken vigorously for 1 minute, then allowed to separate. The blood layer is discarded. The mixture is washed with 10 ml. 0.3 N-NaOH by shaking vigorously for 1 minute, and the NaOH discarded. 10 ml. distilled water are added, the mixture shaken briefly allowed to separate, and the water discarded. The water wash is repeated and the water layer separated as completely as possible. The mepacrine is next extracted into 5 ml. of the acid alcohol mixture by shaking vigorously for 1 minute. After it has separated out the acid layer is run off into a centrifuge tube and spun clear. 3 ml. of the clear solution are pipetted from underneath the separated acum into a non fluorescent test tube. Alkaline borate buffer (0.5 ml.) is added, and the fluorescence compared with standard solutions freshly prepared by dilution with acid alcohol from the stock standard, and treated with alkaline borate buffer in the same manner as the unknown.

The fluorimeter requires about 10 minutes to warm up and should be used in the dark.

*Standards for plasma*—The standard mepacrine solution contains 100 mg per litre. A dilute standard solution is prepared by diluting 10 ml. of the strong standard to 1 litre with 0.1 N-HCl. This solution should be prepared freshly for use. Fluorescent standards are prepared as follows: 0.1 ml., 0.2 ml., 0.4 ml., 0.8 ml. and 1.2 ml. dilute standard are each diluted to 5 ml. with the acid alcohol mixture, 3 ml. of each of these is treated with 0.5 ml. NaOH borate buffer. These are equivalent to plasma of 10  $\mu$ g, 20  $\mu$ g, 40  $\mu$ g, 80  $\mu$ g and 120  $\mu$ g per litre respectively.

*Standards for whole blood*—The same standards as those for plasma are used, but since 5 ml. whole blood are used instead of 10 ml. plasma the standards are equivalent to two times their stated concentration for plasma, i.e., to 20, 40, 80, 160 and 240  $\mu$ g per litre. Additional standards of intermediate strengths may be prepared if it is desired to match the blood strengths more closely. For very high mepacrine concentrations 2 ml. of whole blood may be used instead of 5 ml.



*The Fluorimeter*—This instrument is used on a 220 to 240 A.C. circuit. It should be turned on about 10 minutes before use. The blood fluorescent tube is placed between two standards for matching. The tubes may be viewed vertically by placing in the holes of the rack provided, or they may be viewed horizontally by removing the rack and placing the tubes in the well.

Once the lamp has been switched off it must not be used again until it has cooled down. It should, therefore, be kept on continuously until each set of matchings has been completed.

Suitable lamps are the "Osira" 80-Watt black glass lamp 230 volt, with choke as G.E.C. leaflet 05 7196 or Philips' MBW-V "Philora" ultra violet mercury lamp with lampholder and choke.

Paraffin extraction with measurement in Lovibond Nesslerizer or all-purpose comparator.

*Principle of Method*—The mepacrine is extracted from alkaline urine into paraffin, washed with NaOH, taken into N HCl and the colour measured against yellow discs in the Lovibond Nesslerizer or in the all purpose comparator.

The procedure is an adaptation of previous methods and an attempt to conform to the general principle of methods laid down by MASEN (1943) and by BRODIE and UDEGRIEDEN (1943).

**Apparatus**—200 ml. separating funnel 50 ml. graduated cylinder Low bond Nesslerizer or all-purpose comparator with mepacrine disc, test pipettes. Paraffin, 10 per cent. NaOH  $\backslash$  HCl.

**Method**—To 50 ml. urine in a separating funnel, add 2 ml. 10 per cent. NaOH and about 100 ml. paraffin. Shake well, allow to separate, drain off as much urine layer as possible. Wash by shaking with 10 ml. 10 per cent. NaOH and draining off (The shaking and separating can be done in medicine bottles, by decanting and sucking off but a separating funnel is more convenient.) If the colour is to be read in the Nesslerizer add 50 ml.  $\backslash$  HCl if it is to be read in the comparator add 18 ml.  $\backslash$  HCl. Shake well allow to separate and drain off as completely as possible into Nesslerizer tubes or comparator cells, compare with coloured disc. If globules of paraffin remain suspended in the HCl extract and make the reading of the colour difficult, the extract should be passed through a filter paper. If the colour is too strong dilute 1:1 with  $\backslash$  HCl and re read.

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 MASEN, J. M. (1943). *J. med. Chem.* 148 529.

Mr K. B. Williamson

1. Photomicrographs of sporozoite-containing stomach cysts of a laboratory bred female *Culex bitaeniorhynchus* photomicrograph sporozoites of another similarly infected laboratory bred female of the same species photomicrograph of Ross's black spores in another laboratory bred female of the same species experimentally infected with *Plasmodium citreus* and drawing of the same showing association of the black spores with sporozoites and disintegrated stomach cysts.

2. Photomicrograph of a bladder of *Utricularia* showing captured anopheline larva, and the trigger mechanism discovered by LLOYD by which captures are effected.

3. Coloured photographic transparencies showing brick red water-bloom produced by *Euglena haematodes* after rotting of cut reeds in rice fields in Penang, shown in accompanying photograph, a procedure antagonistic to the breeding of malaria-carrying mosquitoes (WATSON M. 1921 *Prevention of Malaria in Malaya* pp. 213 and 363).

4. Model of an automatic sluice-gate and photographs by Colonel J. W. SCHARFF and the exhibitor (K. B. WILLIAMSON) of larva-control by *natura-fistae measures* including water splashing herbage packing, palm-leaf covering of ditches and wells, and anti-larval sluicing.

ORDINARY MEETING  
of the Society held at  
Manson House, 26, Portland Place, London  
on  
Thursday, 17th May, 1945, at 8 p.m.

THE PRESIDENT  
SIR HAROLD SCOTT KCMG MD FRCP FRCS E  
in the Chair

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PAPER

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RECENT RESEARCH ON KALA-AZAR IN INDIA.

BY  
H. E. SHORTT C.I.E., M.D. D.Sc. COL. I.M.S. (ret.)  
*Reader in Medical Parasitology, University of London.*

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HISTORICAL.

The history of research on kala azar may conveniently be divided into three periods. Firstly there is the period during which serious epidemics of fever were described in Bengal before the actual causation of these fevers was known. Secondly there is a middle period, commencing with the discovery of the parasite of kala azar and leading to the earlier work on the morphology of the parasite and the pathology of the disease and thirdly there is recent work dating from the period when effective means of treatment were discovered and interest was aroused in the problem of transmission of the disease. As the account I am giving tonight deals chiefly with this third period I shall deal with the first two very briefly but they must be considered as the work done during the earlier periods led directly to the more recent developments in research on kala azar in India.

*Early Period*

The earliest records of epidemic fevers occurring in Bengal, and showing a high mortality date from epidemics occurring in Jessore one of the central districts of Bengal about the years 1824-25. In the Burdwan district, to the south-west of the province, a similar epidemic raged between the years 1850 and 1875. About the same period a fever with a very high mortality was epidemic in the Rangpur district in the north-east of Bengal, and from here the disease spread eastwards into the neighbouring district of the Garo Hills in Assam.

The descriptions given of these various epidemics by contemporary writers, such as ELLIOT and FRENCH leave little doubt that some at least, of these outbreaks were kala azar. The possibility that epidemics of malaria may also have occurred over a period of so many years cannot be ruled out, and indeed, such outbreaks were highly probable.

The high incidence and fatal character of the fever as seen in Assam after its spread from Bengal, made necessary its investigation with a view to discovering its cause and, if possible, effective treatment. It was studied, therefore, in this province by ROGERS in 1896, ROSS in 1899 and in 1904 by BENTLEY. These workers were unable to differentiate the condition as a specific disease and considered it to be either a specially severe and intractable form of malaria or malaria complicated by other infections, while the possibility was also considered that it might be a disease allied to Malta fever. These investigations may be considered to close the early period of investigations.

*Middle Period.*

The middle period commences with the recording by LEISIDIAN in 1903 of the discovery of a parasite in the tissues of a case of what was then known in Bengal as Dum Dum fever. His material was postmortem material from a case of the disease which had died in 1900.

In the same year (1903) DONOVAN independently obtained the same organism in the spleens of living cases of the disease. On the publication of his discovery CHRISTOPHERS was immediately deputed to work on this newly discovered parasite, and in 1904 and 1905 he published his classical work on the pathology of kala azar. In 1904 BENTLEY recorded the parasite from Assam, and so established it as the cause of the fever in that province, which was causing such havoc, and was locally known as kala-azar. At the same time the parasite was recorded by CASTELLANI from Ceylon but this observation does not seem to have been repeated.

In 1904 ROGERS obtained cultures of the parasite, and thus demonstrated the fact that it was a flagellate, which he at first considered to be a trypanosome. This observation was repeated by CHRISTOPHERS, and the flagellate nature of the parasite established.

The next important step in this middle period was the discovery by PATTON in 1907 that the parasite assumed the flagellate form when ingested by the bed bug. This development clearly pointed the way to the probability that the disease was conveyed by an insect vector. MACKIE, who was working on the disease in Assam in 1913, was the first worker to suggest the genus *Phlebotomus* as deserving of study in connection with the problem of transmission. During the whole of the period so far dealt with all attempts at treatment of the disease had failed completely, but in 1915 ROGERS introduced to India treatment by tartar emetic which had been used by CRISTINA and CARONIA in Europe in the treatment of leishmaniasis. This treatment was found to be highly specific for kala azar and from this date up to 1921 the treatment of the condition with the inorganic salts of antimony was widely used and its efficacy established by ROGERS, MUIR and KNOWLES and other workers. This date may be said to close the middle period of research on kala azar so far as India is concerned.

### *Recent Period*

Since the introduction of the disease into the Garo Hills in Assam from the adjoining districts in Bengal the disease had continued to spread slowly eastwards along the valley of the Brahmaputra River. One of these outbreaks was at its height in the Garo Hills in 1882. By 1891 the disease was very prevalent in the district of Nowgong further eastwards, and by 1910 the Golaghat sub-division was already heavily infected. By 1917 the Sibsagar sub-division still further eastwards was involved in the epidemic spread of the disease up the valley. From this date onwards a new epidemic commenced which, in the course of a few years involved the whole of the Sibsagar sub-division while at the same time the whole of the country to the west, previously traversed by the disease was equally heavily infected. These epidemic conditions were not limited to Assam, and serious outbreaks of epidemic kala azar were co-existent in Bengal, Bihar and Orissa. The state of affairs was so serious and was having so profound an effect on the economic life especially of Assam, that the Government of India felt impelled to institute a full scale enquiry into the causation and prevention of this disease which in a short period of about 5 years during the height of the last epidemic in the Nowgong area, had caused one fourth of the arable land to go out of cultivation, and one fifth of the population to perish.

I have now brought the story to the period about the years 1921-22, and from this time onwards very widespread and intensive research has been carried out in India on all aspects of the kala azar problem—epidemiology, transmission, treatment, diagnosis and prevention. It will not be possible for me in the time at my disposal to deal anything like fully with all these aspects, some of which I shall, therefore, only touch upon in order to deal more fully with the question of transmission of kala azar, which has been



the subject of the greatest amount of research work done in connection with this disease.

### EPIDEMIOLOGY

There is a considerable literature on the epidemiology of kala-azar but I shall confine myself for the sake of those persons not familiar with this subject to a very brief statement of the history of kala azar epidemics. So far as we know the north-eastern part of India is the only place where very well marked epidemics of the disease have occurred. The condition in most other parts of the world where the disease exists takes the form of a higher or lower grade endemicity. The trend of events when kala azar attacks a new area is best exemplified by Assam. This province, previously exempt from this disease, became infected somewhere about the year 1873 by extension of the disease from the neighbouring districts of Bengal, Dinajpur and Rangpur which are separated from the Garo Hills in Assam by the Brahmaputra River. The Garo Hills area was devastated by a severe epidemic, which was at its height in 1882, and by 1891 the disease was prevalent in the Nowgong district. Between 1891 and 1901 this district suffered so severely from epidemic prevalence, and the destruction in life and the loss of productivity to the area was so great that the words kala-azar from that date have always struck terror in the minds of the people of Assam in all districts where the disease has appeared. I have already mentioned the extent of this destruction to life and agriculture.

The inter-epidemic period, commencing about 1901 lasted until 1915 but the disease kept up a steady insidious advance along the valley so that by 1907 or 1908 the Golaghat sub-division was infected, and by 1910 the disease had reached the Sibsagar sub-division. About 1915 there commenced the most recent of the series of epidemics, which may be said to have come to an end about 1927 or 1928. In the case of a disease where there is a considerable prevalence even during inter-epidemic periods, the exact dates of the subsidence or recurrence of epidemics are not easy to demarcate but, as a general statement, it may be said that since the disease first appeared in Assam there have been periods of 10 years epidemic prevalence followed, in each case by inter-epidemic periods of 15 or 20 years. If history is to repeat itself the time is now due for the occurrence of a new epidemic, and if this is to follow the course of previous epidemics it is likely that new country in an easterly direction, will be implicated. This would mean the invasion of the hitherto free territory of the Lakhimpur sub-division. The only thing I am aware of which might make any difference, as compared with the course of events in the past, is that during the last epidemic we had available an effective treatment for the disease where none existed previously. The application on a large scale of this treatment during the 1915-27 epidemic undoubtedly saved a very large number of lives and, which is more important,

greatly restricted the number of new infections. This was brought about by the fact that early in treatment the parasites disappear from the peripheral blood and such persons are no longer sources of infection. An interesting point to be noted is that the widespread institution of treatment was not accompanied by any shortening of the duration of the epidemic, which declined in areas where treatment had not been instituted at the same time as in those areas where treatment had been pushed.

### *Prevalent forms of the disease*

The disease may be said to exist in two forms. Firstly there is the typical form where the patient suffers from a long continued fever which may have a rapid or slow onset, which is accompanied by great enlargement of the spleen and sometimes of the liver and ends when untreated in death in a very large percentage of cases. Secondly there is the dermal leishmanoid form in which a case of kala azar which has been treated, later develops a condition in which the parasite gives rise to various lesions in the skin while the internal organs appear to be free of the parasite. This condition may sometimes occur in the rare cases which become cured without treatment. This form of the disease was first described by BRAHMACHARI in 1922, since when a great number of cases of this condition have been brought to light, especially in Bengal, but the condition is found also in Assam and in Madras.

### THEORIES IN CONNECTION WITH THE TRANSMISSION OF KALA-AZAR.

This part of the subject I must treat somewhat more fully as the bulk of research work has been directed towards the discovery of the method of transmission of the disease. In previous papers I have classified the various theories under certain heads and I still find this the most convenient means of describing the work done in India, so I will again describe these theories shortly in the same way. The heads I refer to are —

- (a) Transmission not requiring an arthropod vector
- (b) Transmission requiring an arthropod vector and
- (c) Other possible methods of transmission.

#### (a) TRANSMISSION NOT REQUIRING AN ARTHROPOD VECTOR.

Put in its simplest form, this means transmission by contamination, which may be either direct or indirect.

#### *Direct Contaminative Transmission*

For successful transmission by this means two things are necessary first, that the parasite should be excreted from the body of the infected person in viable form, and secondly that this form should be capable of producing infections in a new host through some of the mucous surfaces of the body in

the mouth, nose or conjunctiva. Both these conditions necessary to direct contaminative transmissions, have been proved to exist. Thus, it has been shown that the parasites of kala-azar are excreted in viable form in the urine and in the nasal secretions by successful culture in the first case, and in the second case by infection of animals by inoculation with nasal secretions. The second condition has been fulfilled by the proof that the parasites of the disease can produce infection both when administered by the mouth and when placed on the conjunctiva and, in addition there have been a few experiments where the disease appears to have been successfully transmitted by keeping healthy animals in prolonged close contact with infected ones.

In 1935 FOREMAN and ZIA, working in China, showed that *Leishmania donovani* was frequently present in the nasal secretion of cases of kala azar and that such parasites were viable. This observation was repeated in 1935 by the author and SWAMINATH for Indian kala azar and again it was shown that the parasites were viable by the successful inoculation of a hamster. Here, then is a possible method of direct contaminative transmission where, in sneezing or coughing droplets containing the parasites might be deposited on the conjunctiva or on the nasal or oral mucosa.

#### *Indirect Contaminative Transmission*

This method would necessitate the excretion from the body of the parasite in viable form and the power to live outside the body for varying periods until introduced into a new host via the mouth or some of the other routes which have been mentioned. The latter of these essential factors, for indirect transmission has been investigated in various ways, such as the viability of the parasite deposited on soil or in milk, and also when exposed to desiccation. The parasite would appear to fulfil the necessary conditions to some extent, since it was found that it would live in whole milk for at least 4 days, while citrated milk may even serve as a culture medium. The parasite deposited on soil, moistened by water or urine, lived for 24 hours. Drops of culture containing the parasite, when dried on a coverlip were able to stand desiccation for 4 hours.

#### (b) TRANSMISSION REQUIRING AN ARTHROPOD VECTOR.

The fact that the parasite of kala-azar circulates in the peripheral blood, and that when ingested by certain insects it develops into a flagellate, made it obvious that the possibility of transmission of the disease by some blood sucking insect had to be investigated. PATTON initiated such researches when he discovered that *Leishmania donovani* flagellated in the midgut of the bedbug (*Cimex* sp.) Various other insects have been investigated in this respect, such as the genera *Cororhynchus* and *Phlebotomus* various species of mosquitoes, *Culiscoides* and other arthropods such as lice and ticks. The only instances in which *Leishmania donovani* is found to flagellate regularly is when it is taken

up by *Cimex* and *Phlebotomus*. The large amount of research expended on the bedbug led to nothing but discouraging results and the only arthropod which has given results which I think may be considered conclusive is the sandfly. It is in connection with this insect that most of the work relating to transmission of the disease has been done in India, as well as in other countries where the disease is prevalent. Since the subject of this paper is recent research in India I will confine myself to the work done in that country.

### *Sandflies and Transmission of Kala-azar*

The only species of the genus *Phlebotomus* which has given any indication of connection with kala azar transmission in India is *Phlebotomus argentipes* and any reference I now make to sandflies may be taken as referring to this species.

#### *History*

There is no doubt in my own mind that the first worker who dealt with *Phlebotomus argentipes* in connection with kala azar was MACKIE in 1915. He was working on kala azar in Assam and among other insects dissected sandflies (unidentified) although he made little progress as regards investigating transmission of the disease. He almost certainly dissected *Phlebotomus argentipes* and I am led to this conclusion by the fact that he mentioned finding in his sandflies a peculiar body which I am able to say was one stage of a gregarine parasite, which occurs in *Phlebotomus argentipes*. He also made the observation that sandflies were the only insects which would repay further research in connection with kala azar transmission. Nothing more seems to have been done in this connection until in 1922, 1923 and 1924 in Assam, the author and SWAMINATH dissected sandflies now for the first time definitely identified as *Phlebotomus argentipes*. These flies were caught in kala azar houses, and were found to contain mammalian blood. The results of this work were not published until 1924, when the work was described in a paper delivered at a meeting of the Assam and Northern Bengal branches of the British Medical Association in Assam.

Although the actual record appears to be lost, there is no doubt that in 1922, SINTOV in a private communication to KNOWLES pointed out that there appeared to be some correlation between the distribution of kala-azar in India and that of *Phlebotomus argentipes*.

Later in 1924, the sandfly was finally brought into the limelight by the publication of a paper by KNOWLES, NAPIER and SMITH, in which the fact was established that *Leishmania donovani* when ingested by *Phlebotomus argentipes* assumed the flagellate form.

It was at this stage that the alarm caused by the serious epidemic of kala azar raging in Assam convinced the Government of India of the necessity

for a really comprehensive study of the causation of this disease. The result of this decision was the appointment of the Kala Azar Commission, composed of CHRISTOPHERS as Director, SHORTT as Protozoologist and BURRAUD as Entomologist. The Commission was to work in Assam, and an ancillary enquiry was constituted to carry on work in Calcutta. The members of this enquiry were KNOWLES, NAPIER and other members of the staff of the School of Tropical Medicine in Calcutta. The Kala Azar Commission functioned as constituted until 1926 when CHRISTOPHERS was recalled for other duties, and SHORTT became Director of the Commission. The latter was strengthened by the appointment of CRAIGHEAD and later of SMITH and KRISHNAN. Almost immediately following the appointment of the Commission they were able to confirm the flagellation of *Leishmania donovani* in the gut of *Phlebotomus argentipes* and went on to describe the extension of the flagellates into the pharynx of the sandfly. Every hope was now entertained that the demonstration of the transmission of kala azar by the sandfly would be quickly obtained, but the actual course of the researches which followed has shown how far this was from being the case.

The first point investigated was the life history of *Leishmania donovani* in the insect, and this was fully worked out by 1926. The details of this, as well as the presumed course of events in the human host, are indicated in a diagram shown on the lantern-slide. The life-history fully confirmed the opinion that animal experiments should now quickly demonstrate transmission of the disease by the bite of the sandfly. The only difficulty likely to arise in demonstrating this was the fact that no very suitable animal was at first available, because of the relatively small susceptibility of most of the usual laboratory animals to small doses of parasites. This difficulty was overcome, however, when SARTY and YOUNG (1924) discovered the great susceptibility of the Chinese hamster when experimentally infected. A very large series of experiments with animals was instituted, and to show the extent of these experiments I give a few figures: thus 243 mice were fed upon 9490 times, and thirty-five hamsters 3,358 times, by sandflies previously fed on kala-azar cases. After all these experiments, conducted over a period of over 4 years, in only one case was kala azar transmitted to an experimental animal. This was in the case of a Chinese hamster which had been fed upon 144 times by *Phlebotomus argentipes*. The animal was examined 511 days after the commencement of the experiment.

Previous to this successful transmission, the failure of work with experimental animals had led us to the use of human volunteers, a step rendered justifiable, in spite of the dangerous nature of the disease, by the discovery of a very effective method of treatment. Without going into full details of this experiment it will be sufficient to say that in all eleven human volunteers were fed upon 11,537 times by flies which had previously been fed on kala-azar

cases and refed two or more times to allow the infection fully to develop. These human experiments were entirely negative in spite of an intensity of feeding much greater than would ever occur in nature.

By the time these experiments had been completed, in 1931 the epidemic of kala azar had declined. This and the disappointing results of the experiments with human volunteers led to the decision to close down the Kala Azar Commission until the possible occurrence of another epidemic which would render available enough material in the way of kala azar cases to renew investigation on the method of transmission with all the information gathered in the course of 7 years study available.

In order to lead to the reasons which caused me to renew the work on kala azar transmission by the sandfly I should say a few words on the bionomics of the sandfly and describe the actual technique employed in the experiments which have already been mentioned.

#### Bionomics of *Phlebotomus argentipes*

When this insect first came into prominence in connection with the transmission of kala azar very little was known about its life history. The result of this ignorance was soon apparent when experiments with it came to be carried out.

It was found that these flies when fed on blood, deposited their eggs 4 or 5 days after the blood meal and then almost invariably died. This fact held up attempts at transmission of the disease by this fly for a long time and it was only after the discovery of the conditions necessary to its continued life after oviposition that experiments in transmission by infected flies could be carried out. The flies seldom survived oviposition except at a temperature of 28° C. or 1 or 2 degrees above or below this. These are the conditions which actually prevail in the hiding places of this insect, in cracks or crevices throughout a considerable part of the year in Assam.

Adult flies are found in nature throughout a great part of the year in Assam and North Eastern India, but they are absent during the coldest months of the year from December to February. Survival of the flies between one season of prevalence and the next most probably occurs chiefly, if not entirely, in the larval stage. Only the female sucks blood and such a blood meal is not only necessary for the maturation of the batch of eggs but also for the development of a fresh batch of eggs after each oviposition. Fertilization of the female sandflies occurs soon after hatching and one act of fertilization appears to suffice for the whole subsequent life of a fly. The midgut of the adult fly is normally sterile so far as bacteria are concerned, and it is this fact which allows the development of *Leishmania donovani*. In this situation infection of the gut with bacteria seems invariably to result not only in the destruction of flagellates but in the death of the fly.

### Feeding Experiments

The sequence of events in carrying out any particular experiment on transmission by the sandfly was as follows —

Laboratory bred sandflies were fed on a case of kala-azar showing parasites in the peripheral blood. These flies were then kept at a temperature of 28° C. for 3 or 4 days, during which time the blood meal was digested and the fly's ovaries developed fully. On the 4th or 5th day the fly oviposited and was ready for a second meal. This second and subsequent meals, at intervals of 2 or 3 days, are essential for the continued life of the fly and the meals were either on the kala-azar case supplying the original parasites, on another kala-azar case or most frequently on an experimental animal—rabbit or mouse. It was found that complete development of the flagellate infection in the sandfly had taken place by the 7th to 9th day after the original feed. The infected sandfly was then put on the animal or human being we wished to infect at its third or subsequent feed.

This was carried out for some years on the scale I have already indicated without success except in the one case I have mentioned, and the fact that in no case was a human infection obtained left unforged a very obvious missing link in the chain of transmission before one could say with confidence, that the sandfly was the vector of the human disease.

In 1940 SMITH HALDER and AHMED working on kala azar in Bihar were carrying out transmission experiments with *Phlebotomus argentipes* on lines similar to those I have described above, but they eventually developed a technique for keeping sandflies alive, after their initial feed, by feeding them on fruit juice instead of blood. The principle followed was the same as that used in the keeping alive of mosquitoes after their blood meal. By using this technique, SMITH and HALDER were able to keep alive a considerable percentage of their flies, after only one blood meal, for 7 or 8 days by which time there was a heavy infection of flagellates in the midgut and pharynx. These flies were then given their second blood meal on the animal it was proposed to infect. By this means they were able, in several instances, to infect hamsters and mice.

The development of this feeding technique made me decide to make one more effort to infect human volunteers, using the method of keeping flies alive by feeding them on fruit juice in the interval between their first blood meal and the meal on the volunteer it was intended to infect. The technique for keeping the flies alive has been fully described by SMITH and HALDER, and I need not go into details of it except to say that the fruit used for feeding the flies was dried raisins.

In the new series of experiments which were now started using this technique five human volunteers were used. These volunteers came from a non-endemic area, and continued to live in that area except on the days on which they were required for feeding experiments. They were selected after

a very strict medical examination, including X-ray and serological examinations. In all respects, except that the sandflies were fed on fruit juice instead of blood, the experiments were similar to those which had previously been carried out with human volunteers. The present series however gave us the astonishing result, considering past failures that out of the five volunteers used, every one became infected with kala-azar. It is difficult to get away from the supposition that this result was due to the feeding on raisins this being the only factor differing from those present in previous work on the same lines. However, we have no idea, at present, as to what difference this alteration in diet made to the infectivity of the flies. SMITH was of the opinion that this technique caused the oesophagus and pharynx of the flies to be blocked with parasites as the result of the heavy flagellate growth. This however had often been seen in flies fed on blood and was fully described by the speaker BARRAUD and CRAIGHEAD in 1926. This condition is shown in the lantern slide.

In a first attempt to see whether there is any difference in series of flies fed by the old and the new techniques, comparative batches fed initially on the same kala azar case, and at the same time, were kept apart, one batch being re-fed on raisins and the other batch on blood. So far as the experiments have gone there seems little if any difference in the two series but the results of these experiments have not been published as yet, and they are far from complete. Both the intensity of the infections and the number of flies infected showed no significant difference in the small series so far experimented with. Whether the feeding on plant juices produces some difference in the pH of the gut contents or some other difference which makes the flagellates more infective I am not in a position to say but in face of the evidence this would appear to be so and the point is one which might be worthy of combined investigation by protozoologists, entomologists and chemists.

In a recent issue of the *Indian Medical Gazette* MALONE and BROOKS (1944) have brought up the question of transmission of kala azar by the sandfly once more, and the whole of their paper is devoted to an attempt to show that not only is the evidence for sandfly transmission of the disease inadequate but that the sandfly could not possibly be the vector of kala azar. It is curious that these two workers who so far as I know have never done any work themselves on the transmission of kala azar should attempt a destructive criticism of all the work done by others in proof of such transmission and in fact they lay themselves open to a complete refutation of their assertions were one inclined to initiate a contest in polemics. This is neither the time nor the place to indulge in this recreation, but the article is recommended for study to those interested in kala azar transmission, with the personal opinion that so far from disproving transmission by the bite of the sandfly the paper has merely proved the danger of treading on ground with which one is unfamiliar.



## (c) OTHER POSSIBLE METHODS OF TRANSMISSION

The only other possible method of transmission which has received any attention, was the possibility that ankylostomes might play a part in transmission. The fact that the mucous membrane of the intestine sometimes contains *Leishmania donovani* in enormous numbers in the villi, and that these helminths are blood feeders, makes it certain that in feeding they must often ingest the parasites.

Ankylostome infection is exceedingly common in kala-azar areas, although not necessarily more so than in other areas suitable for such infection but this co-existence at least raised the possibility that the helminth parasite might play some part in transmission of the protozoal parasite, however little the life history of the ankylostome would appear to make it suitable for such a role. All work however done in this connection has given entirely negative results.

Before leaving the question of transmission, I may mention here although I have not been able to deal with it fully in my account, the various possible methods by which infection of experimental animals can be produced. This will indicate how complex the problem of transmission was found to be in the earlier stages of research. All these methods, which are set out below had to be fully investigated before one came to the present, and I think generally accepted, idea that transmission by the bite of the sandfly is probably the final solution of the problem of transmission. Transmission of kala azar has been produced in all the following ways —

- (1) By inoculation of the flagellate and non flagellate stages of *Leishmania donovani* through various routes.
- (2) By intraperitoneal inoculation of the naso-pharyngeal secretion of kala azar cases.
- (3) By feeding on emulsions of liver or spleen containing *Leishmania donovani*.
- (4) By feeding on cultures of *Leishmania donovani*.
- (5) By feeding on the faeces of infected animals.
- (6) By close and prolonged contact, under insanitary conditions, of healthy and infected animals.
- (7) By feeding animals on sandflies containing *Leishmania donovani* with which they had been infected by artificial feeding on liver and spleen emulsions.
- (8) By the contamination of the conjunctiva with infective material.
- (9) By intraperitoneal inoculation of emulsions of *Phlebotomus argentipes*, fed on kala azar cases.
- (10) By the intraperitoneal inoculation of emulsion of *Cimex* sp fed on kala azar cases.
- (11) By the bites of *Phlebotomus argentipes* infected by feeding on kala-azar cases.

## TREATMENT

I have already remarked that until the beginning of the last great epidemic of kala-azar there was no effective treatment for the disease and the mortality from it was probably around 90 per cent. of diagnosed cases. The fact that this state of affairs has been altered to the extent that the mortality of treated cases can now be lowered to a figure round about 5 per cent. gives some indication of the success which has attended research on therapeutic methods.

The first notable advance was the use of antimony salts by VIANNA in 1913. Two years later ROGERS introduced for Indian kala azar the treatment already used by CRISTINA and CARONIA in which disease it was found most effective. The salt first used was potassium antimony tartrate but this was later superseded for the most part, by the sodium salt which was found to be less toxic and more easily tolerated. KNOWLES (in 1918) carried out a valuable series of trials of these remedies, and laid down standards for the dosage and methods of administration of these antimony salts. The new method of treatment, while a great advance, and while resulting in a great saving of life was still a tedious one. The cure occupied 3 or 4 months after which a considerable number of cases were found to relapse, while others proved resistant to the drug.

The next step in treatment was as great an advance as the original introduction of the antimony salts. This was the preparation, in 1922, by BRAHMIACHARI of a pentavalent compound of antimony to which he gave the name urea stibamine. This preparation came into prominence after trials carried out by the present speaker, and SEN from 1922 onwards. The result of these trials was to show that the new preparation was enormously more effective than the salts of antimony previously in use, and the treatment of the disease was cut down to fewer weeks than it had previously occupied months.

The success obtained all over Assam and Bengal with this preparation led to the introduction of various other compounds, which are either identical with or very similar in composition to urea stibamine.

The next composition which was extensively used, was Von Heyden 471 also known as stibosan, which was popularized by NAPIER. A composition said to be an improvement on this was later marketed under the name of neo-stibosan and this also was widely used.

In recent years the therapeutic action of various aromatic diamidines has been investigated in cases of kala azar. ADAMS and YORKE, in 1939 first treated a case of Indian kala azar with diamidino stilbene. The same workers reported the treatment of a second case in 1940. Further cases were treated with this drug and reported on by NAPIER and SEN in 1940. Alarming symptoms in the course of treatment in some of the cases was reported. A further series of 100 cases was reported on by NAPIER, SEN GUPTA and SEN in 1942. The results were said to compare favourably with those produced by neo-stibosan but again the occurrence of troublesome and even alarming reactions was

reported in most of the cases treated. The drug was said to be effective in some cases resistant to antimony.

In 1943 NAPIER and P. C. SEN GUPTA published an account of the treatment of thirty two cases of kala-azar with another of the aromatic diamidines, namely diamidino-di-phenoxy-pentane (M & B 800). They stated that the disease was apparently cured in twenty nine of these cases. The immediate reactions to the injections were similar to those with diamidino stilbene, but were of a milder degree. The danger of too early publication of such results is exemplified by the fact that a follow up by P. C. SEN GUPTA of these cases, over a mere 6 months period, has shown that more than one-third of the cases relapsed.

So far as I know the results given by these later preparations have shown no advantage over the original urea stibamine, and I notice from a recent publication by LOWE, as well as from a conversation I recently had with him, that he is still using urea stibamine for the many cases seen by him in Calcutta, and that in his opinion it is as effective as any of the newer preparations.

Besides the standard treatment with urea stibamine lasting for 2 or 3 weeks, various methods of intensive treatment, where the drug is given at much more frequent intervals, have been tried and are, apparently often enough to produce a cure. It is difficult to see how any future treatment can show any marked improvement on that now available in these antimony preparations, where the cure of a disease, for which previously there was nothing to prevent a fatal termination, can be obtained sometimes in a matter of days.

### CLINICAL DIAGNOSIS.

As I am dealing chiefly with more recent work I need not go into the question of the differential clinical diagnosis of kala-azar since I have nothing to add to what is found in the textbooks. The only remark I would make is that the disease most likely to cause a confusion is infection with one of the enteric group. The usual mode of onset is very similar in all these diseases and the fact that there are aberrant cases in them all, such as those with a sudden onset, does not make the clinical diagnosis any easier. We must, therefore, have recourse to laboratory methods for a definite diagnosis. I shall not do much more than mention these laboratory methods as they also are to be found in the textbooks, but a few remarks on some of them may not be out of place. The more important of these methods are given below.

#### *Methods of Direct Examination*

(a) Spleen or liver puncture with microscopical examination of stained smears from these organs. This is the method of choice for the great majority of cases.

(b) Sternum puncture, and examination of stained smears of the bone marrow extracted. This method I consider less reliable at least than spleen puncture, but it would be the method of choice in the case of a postmortem performed a considerable time after the death of the subject or experimental animal. This is because the parasites after death seem to disappear as the result of decomposition more rapidly from the organs than from the bone marrow.

(c) Microscopical examination of the peripheral blood. This, in the hands of an experienced worker, will give a positive result in 80 per cent. of cases.

### *Cultural Methods*

(a) Culture of the material obtained by spleen, liver or sternal puncture. This is probably the most certain of all methods of obtaining a definite diagnosis.

(b) Culture of the peripheral blood. This method will give a positive result in over 90 per cent. of cases of kala azar.

### *Xeno-Diagnosis*

This is merely an academic procedure whereby one produces a flagellate infection in *Phlebotomus argentipes* fed on the peripheral blood of the case.

### *Chemical Diagnosis*

Various tests have been employed in which chemical agents are brought in contact with the serum of kala-azar cases. It has been shown by LLOYD and PAUL (1928) that in kala-azar there are marked changes in the composition of the serum, the most important of which is a great increase in the total serum globulin, and the euglobulin fraction and an absolute decrease in the serum albumin resulting in a great raising of the globulin albumin ratio. It is presumed that this is the reason for some of the reactions to be mentioned. The first of these was —

(a) The globulin precipitation test of BRAHMIACHARI described in 1917. In this test the serum of a kala azar case is mixed with twice its volume of distilled water. A marked precipitate indicates a positive test.

(b) SPACKMAN in 1921 introduced a modification of the formol gel test used by GATÉ and PAPACOSTA in syphilis. This test, or modifications of it, was later popularized by NAPIER.

(c) CHOPRA, GUPTA and DAVID in 1927 introduced the urea stibamine or antimony test, later modified by various workers.

### *Complement Fixation Test.*

Early attempts to devise a complement fixation test for kala azar were not very successful. Little interest in this means of diagnosis was taken in

India until comparatively recently. NIYOGI and RAY (1942) reported satisfactory results in a small series of cases of kala azar using an antigen prepared from *Leishmania* cultures. More recently the most encouraging results have been obtained, curiously enough, with the use of an antigen having no relationship with the parasite of kala-azar. This antigen is that originally prepared by WITERSKY, KLINGENSTEIN and KUHN from the human tubercle bacillus. This test was devised for the diagnosis of tuberculosis but the results were poor and it is curious that it should now apparently prove of great value in the diagnosis of kala-azar.

The first worker to use this test seems to have been BIER in cases of South American leishmaniasis. These results were confirmed in India by LOWE and GREVAL in 1939. By using this, or some modification of this test, it soon became evident that a valuable aid to the early diagnosis of kala azar had been discovered. In a report on a series of 434 cases, coming for diagnosis, SIKH GUPTA (1943) obtained a positive reaction in 172 out of 177 cases diagnosed as kala-azar (97 per cent.). A large number of control cases gave negative results.

The reaction apparently becomes positive very early in the course of the disease for instance, within 4 weeks of the onset of illness. The preparation of the antigen for this test presented some problems owing to difficulty in obtaining adequate quantities of cultures of human tubercle bacillus. The test was, therefore, modified by using an antigen prepared in the same way from KIDROWSKY's bacillus, which gives a luxuriant growth on glycerine broth in 3 weeks. The technique adopted was essentially the same. The number of cases investigated with this new antigen was over 900 including cases of all kinds of diseases. The results of this particular investigation have been reported on as follows —

The number of cases investigated was 920. In cases otherwise proved to be kala-azar a positive reaction was obtained in 93 per cent. of cases. A doubtful reaction was obtained in 6 per cent. and a negative in 1 per cent. Of all cases likely to be considered in the differential diagnosis of kala azar 99 per cent. gave a negative reaction, while 1 per cent. gave a doubtful reaction. A positive reaction was given by a small proportion of obvious cases of chronic pulmonary tuberculosis. This test gave a positive reaction in some cases of kala-azar within 3 weeks of the onset of illness.

Such early cases are always negative to the aldehyde and antimony tests. It will be seen here that the percentage is lower than that obtained when the original antigen was used, but it seems evident that this test will be of value in the diagnosis of early cases.

#### Prevention and Control.

If we accept the sandfly transmission of kala-azar there is no easy way to control the disease, except by attacking the adult stages of the sandfly

Attacking any organism in the ground and it is here that the sandfly breeds is difficult. On the other hand the sandfly is a delicate insect and very vulnerable to insecticides which can reach it. Its habit of going into minute cracks and crevices in houses or in the immediate neighbourhood outside them would make it necessary to bring the insecticides into these situations but any attempts so far carried out have been tentative and on a small scale. There is no reason, however, to believe that if the newest methods used against other insects were continuously and conscientiously applied throughout the sandfly season that they would not have a large measure of success.

The only other preventive measure which undoubtedly had great success during the last epidemic in Assam, is paradoxically enough treatment of the disease. Very early in the course of treatment it is found that parasites disappear from the peripheral blood, and the case is no longer infective to the sandfly. The beneficial effect of this in preventing the infection of new cases may be realized when it is stated that in the peak year of the epidemic in Assam over 60 000 cases were treated and the sources of infection for sandflies proportionately decreased.

### SUMMARY

An account is given of recent research in India on the epidemiology, transmission, bionomics, treatment, diagnosis and control of Indian kala azar.

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## DISCUSSION

The President, Sir Harold Scott You have all heard this interesting and to me, very instructive address. Those who have read Colonel SHORTT's paper will remember that he issues a warning of the danger to those who try to speak without sufficient knowledge, or on ground with which they are unfamiliar. Therefore I will keep away from the question of recent research, but I should like to say a word or two on the historical aspect, which has been more my study than the actual practical side. Colonel SHORTT mentioned in passing about ankylostomiasis. It was the original suggestion made by GILES in 1889 that, whatever it might be elsewhere kala azar in Gauhati where he was working was ankylostomiasis and that it was obviously absurd to attach any pathognomonic importance to the enlarged spleen in connection with the aetiology of kala azar. His reason was that splenomegaly was so common in healthy people. He thus fell into one error by the same process as that by which he evaded another. If he had reversed his statement and said that splenomegaly was a symptom of kala azar and the presence of hook-worm was common in apparently healthy persons, he would have been more nearly correct. Investigators in those days were still tied up with the bed bug theory. The bed bug is the most artful of insects. It has been charged with causing disease after disease—kala azar, plague, leprosy and hosts of other things but every time it has just escaped by the skin of its teeth—if it has teeth. It will probably be caught some time. PATTON said not very long ago that the bed bug theory was nearly complete. Colonel SHORTT mentioned the enormous number of 9,490 feeds on mice and 3,358 feeds on hamsters over a period of 4 years with only one positive result. I do not think he mentioned the possibility that infection might be conveyed through the body fluids by the sandfly being crushed on the skin in the act of feeding. Or even by the



faeces of the fly contaminating the skin. I have nothing more to say except to congratulate the Society in having prevailed upon Colonel SHORTT to give this interesting address.

**Maj-Gen Sir John Taylor** I remember well the disappointment that was felt when we had to wind up the Kala-azar Commission without having accomplished a successful transmission to human volunteers by the sandfly and, as far as I recollect, Colonel SHORTT at that time expressed the opinion that this would have to await the occurrence of a new epidemic wave. In the meantime a new technique was developed for retaining the sandfly alive for several successive feeds so that it would reach the infective stage. In the earlier experimental transmission work with hamsters I do not think the very heavy feeding was successful in any large proportion of cases, but by Colonel SHORTT's own endeavours the human experiments were subsequently carried out in Assam with 100 per cent. of successes. The conditions of the experiment were very different from those in which 11,000 sandflies were fed on large numbers of mice, and the point arises as to whether the success in this latter period had anything to do with the particular epidemic phase, or whether it was entirely due to the new technique of maintaining the sandflies. One cannot make a 100 per cent. claim that it has been proved to be the normal method of transmission, but a very prolonged consideration of the other possibilities tends to confirm the opinion that it probably is. Amongst the eleven different methods which Colonel SHORTT mentioned as being tried for transmission the successes were very small, whereas in this case of the five human volunteers it was 100 per cent. It would be very important that we should know to what extent the sandfly in nature has successive feeds and a duration of life corresponding to what were the conditions of the experiment? That I think is of primary importance—we want more information on the point. In India the subject has not been dropped. Now that with this technique successful transmission has been obtained with *Phlebotomus argentipes* we ought to have experiments carried out with the other species of sandflies and, in particular the species prevalent in the areas in China, the Sudan and the Mediterranean where kala-azar also occurs. These, I think, are experiments that would possibly strengthen the case for the sandfly being the normal vector. We are still confronted with many problems of kala-azar particularly the factors that are concerned in the long cycles of epidemic prevalence at varying levels. One might speculate on possibilities relating to virulence and development of immunity but there is nothing at present one can bring forward as likely to be capable of proof in the matter. The question of prevention is certainly one of the most difficult. We know from what has been done on the study of sandflies in India, particularly in relation to sandfly fever that control is very difficult, but the use of new insecticides along with house spraying may be our best line of defence in the future. I was in Assam as Director of

Public Health at one of the most serious epidemic periods when we started extending the antimony tartrate treatment on a large scale this involving two intravenous injections a week for 3 months. It was a trying business for all concerned with the disease scattered all over rural areas with poor communications and it was a tremendous relief when pentavalent compounds came into use. In a period of a fortnight eight injections produced results equal to those obtained with antimony tartrate in 3 months. We are in a better position to deal with epidemics and save lives nowadays but, in the application of insecticidal methods over thousands of villages and rural areas, to get the local people to persist in the work will not be easy. We are still likely to have, in the event of another epidemic wave, a problem of very great magnitude.

**Sir Phillip Manson-Bahr** The explanation of the raisin diet in the transmission of *Leishmania donovani* was certainly an enigma. Under natural conditions it was generally accepted that female sandflies live exclusively on blood, but was it possible that they also had a vegetable feeding stage? Glucose is necessary for the sustenance of malarial plasmodia in culture and also in the blood stream, so might it not be this element which promotes proliferation of leishmanial flagellates in the body cavity of *Phlebotomus*? Regarding the treatment of kala azar experiences in military cases had not always been happy. He had been consulted about one extraordinary instance which had drifted into a hospital in South London after a year's illness during which time it had been regarded as some form of carcinoma. The course had been mostly afebrile, whilst the enlargement of the liver and spleen had been of such a degree as to present the clinical picture of an enormously bloated abdomen to which the spider-like extremities were attached. But the most remarkable feature was the extreme anaemia. The haemoglobin was estimated at 10 per cent. the leucocytes as 400 per c.mm.

Repeated blood transfusions were without effect and there had been no response either to stilbamidine or to antimony gluconate. It really seemed that, in certain circumstances in this war our treasured drugs seemed to go astray. Emetine no longer cured amoebic dysentery quinine was sometimes ineffective in malaria and antimony in kala azar.

**Air Marshal Sir Harold Whittingham** May I take this opportunity of congratulating Colonel SHORTT on the excellent work that he and the Kala-azar Commission have done in India, and compliment him on the excellent sections of the sandfly he has shown us today. One of the chief points he has made is that *Phlebotomus* is probably the insect vector and in this connection it is very important to be able to breed the sandfly in captivity also to extend its life as far as possible for transmission experiments. The first time the sandfly was ever bred in captivity was by the Royal Air Force Sandfly Commission in 1922 and 1923 in Malta and in England. The work was done with *Phlebotomus*

*papatasi*, *perniciosis* and *muscivorus* but the problem was very similar to that with *P. argentipes*. BARRAUD in 1925 before he went out to work in India, also the people who went to do kala azar research in China, came to see the methods of breeding the sandfly carried out by the R.A.F. Sandfly Commission. At that time we were able to keep the sandfly alive for at least 12 days sometimes for 28 days. We showed them how to breed and keep sandflies alive and I think credit is due to the R.A.F. Sandfly Commission for being the first to breed this insect and so prepare the way for the necessary disease transmission experiments. A point has been raised about the frequency of blood feeding of wild sandflies. We collected several thousand wild sandflies in Malta, in barracks, houses, out houses and hen-houses, and found that commonly they had not had a blood feed for days. You could tell roughly the number of days since the last feed by the state of digestion of the blood in the stomach and the residue, and quite a number had not had a feed for about 6 days. This time interval between feeds may help to explain how sandflies in nature live long enough to transmit kala azar.

Dr G. Carmichael Low: I had the same difficulty in keeping mosquitoes alive, when working on filariasis in the West Indies, until I found out that they would thrive on fruit such as banana and fruit juices. The male mosquito does not suck blood so must live on such things. Colonel SHORR solved his problem by using raisins. When epidemics of kala azar are on, the sandflies must be in a condition to infect people easily and if one could get them in the same condition in the laboratory then one should be able to infect volunteers without trouble. Colonel SHORR's work clearly shows that the sandfly is the common infecting agent of kala azar. The destruction of these pests will, however, be no easy matter.

Dr C. A. Hoare: I should like to know Colonel SHORR's views on one epidemiological aspect of Indian kala azar to which no reference has been made in his interesting communication. It is the question of possible reservoir hosts of the infection.

In other types of human leishmaniasis it has been demonstrated that some of the lower mammals serve as reservoir hosts from which the infection spreads to man. The evidence is especially striking in the case of oriental sore which, in rural areas of Russian Central Asia, is a natural disease of desert rodents (especially gerbils) whose burrows are infested by the sandfly vector. In the case of visceral leishmaniasis there is some evidence pointing to the dog as a reservoir host of infantile kala-azar. In fact, it is conceivable that both oriental sore and kala azar represent zoonoses i.e., essentially diseases of the lower animals which are communicable to man.

The epidemiological role of these animals is supported by the fact that they are closely associated with sandflies. In Turkestan these insects breed

all the year round in the burrows of wild rodents which represent the foci from which cutaneous leishmaniasis is disseminated among human beings. As regards visceral leishmaniasis it has been shown that in Tashkent infected sandflies have a focal distribution restricted to places where there is a concentration of dogs.

However, the position regarding the classical kala-azar of the Indian type is quite different, for up to the present all attempts to incriminate dogs as hosts of *Leishmania donovani* in India have failed. Nevertheless it is difficult to admit that this disease, which is so closely related to the other forms of human leishmaniasis should differ so markedly in its epidemiology.

It is possible therefore that Indian kala azar is also a natural disease of some lower mammals—other than dog but hitherto undetected. The position with regard to the two types of visceral leishmaniasis (Mediterranean and Indian) is comparable to that of cutaneous leishmaniasis in Central Asia. While in rural areas the reservoir hosts of *L. tropica* are represented by wild desert rodents in urban districts their place is probably taken by some other animals possibly domestic rodents or hedgehogs.

This problem obviously stands in need of further investigation. The discovery of animals naturally infected with *L. donovani* would be of considerable epidemiological importance. In the first place by revealing the source of human infection it would help to elucidate the distribution and incidence of the disease. In the second place, it would provide a method of prevention and control by attacking the reservoir hosts. This method has already been successfully applied both in the case of oriental sore and in the case of infantile kala azar.

Dr C M Wenyon. On the subject of maintenance of sandflies in captivity referred to by Colonel SHORTT and Sir HAROLD WHITTINGHAM I remember that in Malta in 1913 I was occupied in trying to keep sandflies and in one instance succeeded in keeping a captured sandfly alive for over 40 days (20th June to 5th August). I kept the flies in porous pots covered with gauze and standing in saucers of water. The flies were given an opportunity of feeding on myself every 2 or 3 days. I published a note on my technique but the paper I think has long since been forgotten.\*

Colonel SHORTT has referred to the demonstration by PATTON in India that in the bed bug the parasite of kala azar will develop into flagellates. The observation was regarded by PATTON as proving that the bed-bug was the vector. For a number of years PATTON and I crossed swords over his claims. It appeared to me that the leishmania did not behave in the bed bug as they would be expected to do in a true host. They showed little evidence of extensive multiplication and did not establish themselves as a permanent infection. It was clear to me that the stomach of the bed bug filled with blood was func-

\* WENYON C. M. (1913). *J. London Sch. trop. Med.* 2, 170

tioning merely as a culture tube. I was able to show that other organisms besides the leishmania were able to develop in the stomach of the bug—organisms such as *Trypanosoma lewini* of which there could be no question of the bug being the transmitting agent. This capacity of development in the stomach of the bug has misled many into supposing that the bug is a vector but in all cases the bug has been completely vindicated.

At one time the flea was regarded as a vector of kala azar in the Mediterranean area. In Malta I tested this theory by transferring to two young dogs imported from England about 400 fleas taken off a dog suffering from kala-azar. In a few weeks time the two dogs in a flyproof enclosure began to lose condition and finally died, both on the same day. I found that the 400 fleas had multiplied and that many thousands of fleas had abstracted blood from the dogs to such an extent that they had died of a profound anaemia. At postmortem examination the organs were quite white while the spleen was reduced to a diminutive size. When the dogs were dipped into a solution of lysol to kill the fleas the liquid which ran off their bodies was like pure blood. Needless to say there was no evidence whatever of a leishmania infection. It seemed clear that the Italian workers, who were the chief exponents of the flea-transmission theory had mistaken the natural leptomonaad of the flea for the parasite of kala-azar.

Colonel SHORTT has spoken of the sandflies taking up leishmania from the blood, where of course they do occur. I would like to ask him whether he thinks the sandflies take up parasites from the skin rather than from the blood. They are known to occur in macrophages in the skin as was first shown by CHRISTOPHERS in his most excellent report on the pathology of kala-azar published in India in 1904\*. Again there is post kala-azar dermal leishmaniasis, where leishmania occur in large numbers in the papules characteristic of this condition. Do sandflies infect themselves from such papules and if they do are they capable of transmitting kala-azar? On the subject of post kala azar dermal leishmaniasis, I am reminded of a case of leishmania infection described to me and since published by Captain BURCHENAL† of the U.S.A.M.C. This was in an airman who had served in Sicily whose only complaint was swellings in the neck. Finally diagnosis was established by excision of one of the enlarged glands. In smears of the cut gland definite leishmania were found. There were some slightly enlarged glands in other regions but no other symptoms, while sternal puncture gave a negative result. I wonder what is the explanation of this condition?

I was interested in Sir Philip MANSON BAHR's experience with the drug sodium antimony gluconate which I gave him for the treatment of a case of kala azar. In this case it produced toxic symptoms, which is perhaps remarkable, as it has been used by a number of doctors all of whom report that it is almost entirely non toxic. It has been used by KIRK with some success in

CHRISTOPHERS, S. R. (1904) *Sci. Mem. Off. med. serv. Dep., Gov. India, N.S.*, 8.  
† BURCHENAL J. H. (1945.) *War Medicine* 7 173.

the resistant Sudan cases. The drug is very convenient to use as it is in solution in ampoules ready for intravenous or intramuscular injection. Each ampoule contains 6 c.cm. representing 120 milligrams of quinquivalent antimony. It is issued by Burroughs Wellcome & Co under the name sodium stibogluconate, by Glaxo under the name stibatin, in the U.S.A. under the name stibanose and in the U.S.S.R. under the name solusurmin. It corresponds to the original solustibosan of German origin. Some of the cases treated by Captain BURCHENAL, Dr. KIRK and others have required more than one course of the drug. Accordingly in view of its low toxicity, stronger solutions, containing up to 100 milligrams of antimony per ampoule, are prepared and from reports so far received it would seem that these may give even better results than the weaker solution.

Colonel Shortt (in reply). Some of the omissions in my account which have been pointed out are due to the fact that I have been dealing only with Indian work. Of all the questions asked I fear I can answer only very few. First of all our PRESIDENT mentioned that very many feeds by the presumptive vector may be unsuccessful and he asked whether the crushing of flies on the skin might not be the method of transmission. That has been tried. Experiments have been made to show whether transmission could be produced by that means. I think I am right in stating that the Chinese workers introduced a little tapping instrument to do the crushing and Dr. JOCELYN SMYLY may know if any of these experiments were successful.

The production of infection is merely a question of the introduction of the parasite into the host so that perhaps it could be done in that way.

Sir JOHN TAYLOR put forward some suggestive ideas as regards lines of investigation. He was quite correct in saying that in the earlier experiments with hamsters only a proportion of successful transmissions was obtained whereas in the human experiments there was a 100 per cent. success rate. The question he raised about whether the later success was due to a difference in the epidemic phase is one which we have always considered a possibility. Living through the last epidemic, seeing it rising, attaining its peak and declining, I definitely got the idea, although I have nothing in the way of figures to support it, that the parasite seemed to be more virulent when the epidemic was on the increase and at its peak than when the epidemic began to decline. The reason I say this is that in the earlier and peak stages there was a very large number of acute cases where the patient rapidly developed symptoms of illness and the disease took an acute form, whereas in the later stages of the epidemic there was a larger number of more chronic cases. It looks as though, possibly at the peak period or at the beginning of the epidemic, the rapid transference of the parasite from case to case may have enhanced its virulence. This is only one possible explanation. The fact is that the type of case altered. Another point noticed was that the acute fulminating cases seemed more easy of cure than the

chronic cases at the later stages of the epidemic. As regards the eleven methods given by me whereby infections could be induced, I mentioned that the successes in many of these methods were not small—thus by giving infective material to hamsters by the mouth we could infect in a very large majority of cases. The same applies to experiments on the conjunctiva and so on. Sir JOHN TAYLOR also raised the question of the sandfly in nature—how many successive feeds does it have? We don't know—but we know that the sandfly under ordinary conditions of the laboratory can feed and lay eggs again and again. By an examination of the ovaries of a sandfly coming to feed you can generally state whether it has already laid eggs, and if that is so it has certainly lived 8 or 9 days—because having laid one batch of eggs we presume it has had one previous feed. We cannot say the food was not plant juice, but the only food we can identify is blood or blood serum. I quite agree that having obtained this success by a particular method we ought to apply it to other sandflies. We have already tried other sandflies so far as the old technique with blood was concerned without success. I have no doubt that workers in other parts of the world, as conditions get more normal, will be trying this, and the same would apply to investigations with the similar parasite which is the cause of oriental sore.

I think these remarks more or less answer the question raised by Sir PHILIP MANTON BAHR about whether it is natural for these insects to feed on plant juices. As I say I don't know—but there is no reason why they should not, and the male sandfly probably will feed on plant juices. I have known it in one case feed on blood. I think Sir PHILIP MANTON BAHR'S query quite suggestive as to whether the stimulating factor in the plant juices is glucose. It may be so. As a matter of fact we tried feeding flies on solutions of glucose. It was difficult to keep the glucose sterile and the results were not particularly good. Some of the flies lived and the infection went on but the method did not seem quite so successful as when the fly got its glucose, if glucose it was, from the fruit direct.

The question was raised by Dr CARMICHAEL LOW about mosquitoes feeding on plants. It was the same technique in essentials that we used for keeping the sandflies alive. Nobody suggests that the mosquito feeds on blood and then feeds only on plants until its next infecting meal and there is therefore no reason to suppose that the sandfly does so. It will certainly feed happily on blood if we wish to keep it alive in the laboratory just as on plant juice, and both methods are used. I am glad Dr LOW pointed this out as it may be that some workers attach too much importance to the plant juice meals.

Dr HOARE raised the question of a reservoir host. It was obvious that we must consider this and, so far as work in India was concerned, we considered that point very seriously and examined, naturally first of all dogs, because they had been proved to carry the disease in other countries, or at least to harbour the parasite. We examined also cattle because these are closely associated with human beings. The cattle very often are housed at night under

the same roof as the people in the houses. I was told recently—I have not found it myself—that somebody has described the presence of leishmania in a buffalo

Dr WENYON It was cutaneous leishmaniasis

Colonel SHORTT I do not think we examined buffaloes, but there is no reason why this finding if it was common, should not be repeated straight away and the proportion of animals infected found. It is not a disease of buffaloes, but they may harbour the parasite

In reply to Sir HAROLD WHITTINGHAM I am not sure whether he realized—perhaps I did not make it clear—that the sandflies we were using were practically all bred in the laboratory. We bred many hundreds of thousands of sandflies because it was necessary to have them always available, and the breeding technique had to be brought to a very fine art. We had to devise a means of breeding them in large numbers and keeping them clear of natural parasites, protozoal and helminthic. The whole of the work was done with sandflies bred in the laboratory

Dr WENYON said I did not mention fleas when talking of possible vectors. We never did any serious work with fleas in India in connection with kala azar especially as we knew of his own results in other places. He raised the point whether the sandfly gets the flagellates from the skin or the blood. We have not noticed many cases if any where the human skin is really full of parasites. It has been described in dogs and hamsters but we have not found the condition in humans. Cases of dermal leishmanoid most often occur after treatment of kala-azar but they also occur in rare cases which have been cured without treatment. We have fed sandflies on these and infected the sandflies but whether from the skin or blood I do not know. In one very marked case, where the disease had not been treated and the lesions contained leishmania for a long time we were unable to infect the sandfly from the lesions themselves. We did eventually and then we found that the difficulty was due to the fact that the proboscis of the sandfly did not penetrate deeply enough to reach the parasites

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Dr Edmund Burke (Contribution to discussion submitted after the meeting)

As one who spent 18 years entirely in epidemic kala azar areas in the Assam Valley being medical officer to a large tea estate practice, I trust I have a reasonable excuse for joining in this discussion on Colonel SHORTT's brilliant paper. When I first arrived in Assam in 1926 very newly qualified, and quite a "greenhorn" I fear I fortunately soon became acquainted with the names of those research giants, SHORTT, NAPIER, KNOWLES, ACTON and others but the teaching and work of SHORTT and NAPIER were of special I should say



vital, importance to all in Assam concerned in the fight against kala azar and their success against great odds was always a tremendous inspiration to myself and other humbler workers. I would like to say a few words as regards the sections of Colonel SHORTT's paper which deal with (1) prevention, and (2) treatment.

I have published one full paper on the prevention and control of kala-azar in tea estates\* and one original preliminary note on modern treatment with stibasin (Glaxo)†. Colonel SHORTT mentioned that as treatment with antimony could be so effective, the spread of disease from infected patients could be, by this means, well controlled. In Assam, however during the first 4 years of the war and up to the time I retired (February 1944) no drugs were available often for months at a time for the treatment of kala-azar (or for malaria) and this was during a pandemic of both diseases in the province. Fortunately for most of that time (though not continuously) pyrethrum spray solution was obtainable. In the circumstances I decided to concentrate on prevention by attacking the adult sandflies by two methods (1) spray killing (2) cutting off the enemy's food supplies, his vast plant juice "depôts," which existed in the form of thick jungle of a certain type that abounds in and almost literally smothers tea estates, coolie lines as well, as Colonel SHORTT no doubt vividly remembers. The deprivation of the source of plant juices was done by radical jungle-clearing from every possible place in the estates. In some cases, owing to war time shortage of labour or for other reasons, an alternative method, less attractive to the eye but nevertheless quite effective, was to actually spray the very jungle itself, concentrating heavily on the plants nearest the line houses. This was continuously and thoroughly done till the lines, houses and jungle reeked of insecticide.

Sir JOHN TAYLOR has just remarked that this spray killing method was a very obvious and good one, but whole communities everywhere would not be able to carry it out continuously. With DDT now available—there was none in my time—the effectiveness of spraying will of course be more sustained and marvellously intensified. On well-run and well disciplined tea estates, where large communities of labourers are controllable and control of operations can be maintained fairly easily this spraying method can and should always be carried out year in year out, especially as supplies can be easily maintained for the work in peace time.

As regards treatment of kala-azar I have used practically all the well-known older and new antimonial remedies, but I have never used the aromatic non-antimonial diamidine compounds, e.g., M. & B 744 and M. & B 800 as they are not suitable for simple mass administration in tea estate practice for reasons given in my paper of 1943. I have treated about 8,000 cases of kala azar (1928 to 1943) and, from 1935 nearly 80 per cent. were treated with Bayer's neo-

\* *Ind. med. Gaz.*, January 1943.

† *Ibid.*, June, 1944.

stibosan. Since the war began, after supplies of this compound were exhausted in India, I fell back upon what was still available of solustibosan and finally I was forced reluctantly to revert to urea stibamine many years after abandoning it for mass administration. My personal experience of urea stibamine does not agree with the reported opinion of LOWE, of Calcutta which Colonel SHORTT quoted. In my opinion it is not entirely satisfactory. It is certainly not a quick, pleasant or non toxic treatment, and very careful selection of cases is necessary before use. Young children cannot be treated well with it and this is a serious drawback in epidemic conditions.

SIR PHILIP MANSON-BAHR has told us what a quandary he is in over his most interesting but 'perverse' kala-azar patient. Since the outbreak of war we in Assam were often in dire despair but for a different reason—the lack of the wherewithal to treat our cases, as I have stated.

In my case a heaven sent answer to my fervent prayers arrived at last one day in 1943 when Glaxo Laboratories (Bombay) sent me a most generous free supply of their new product, sodium antimony-V-gluconate or 'stibatin' (Glaxo). I was the first to try it in Assam. The results were astonishingly good in my first series of twenty-one cases treated in collaboration with my chief assistant, Dr K. C. CHAKRAVARTY as described in the *Ind med Gaz* (June, 1944). CHAKRAVARTY has since independently described excellent results in a further thirty two cases and they all conform to the usual accepted criteria for cure. Three cases of the series had had previous courses of ten to twelve injections of urea stibamine. Though resistant to this drug, they responded readily to stibatin. Sir PHILIP MANSON-BAHR's use of Burroughs Wellcome & Co's similar product of antimony gluconate given to him by Dr WENYON should have had better fortune. His patient is obviously a very rare exception. I have had many similarly severe cases, but at least my patients did 'co-operate' when the new stibatin was exhibited. Stibatin, so I believe, is not claimed to differ in any respect from solustibosan, yet the latter produced, in my experience, toxic reactions and a fair relapse rate.

Finally for all the reasons given in my above-mentioned paper of 1944 as well as because of the newer concentrated and higher individual dosage now recommended by the makers—higher than those I originally considered quite bold during my experiments—I consider that stibatin (Glaxo) is as good as any anti-kala-azar remedy ever put on the market, and in my opinion it is superior to many.

In conclusion may I add my congratulations to those already accorded to Colonel SHORTT on his very instructive and exhaustive paper.



## COMMUNICATIONS

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### TSETSE FLY CONTROL AND SLEEPING SICKNESS IN THE SUDAN

BY

A. R. HUNT, L.R.C.P. L.R.C.S.

AND

J. F. E. BLOSS M.R.C.S., L.R.C.P., D.T.M. & H.\*

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### I.—INTRODUCTION

Sleeping sickness (*Trypanosoma gambiense*) has been endemic in parts of the southern Sudan for many years. It has never assumed the serious proportions that it has done in other parts of Africa, but locally it has presented many difficulties. The area around Tembura was the most seriously affected and up to the present the disease has never been eliminated from there.

Control measures in the past were directed to restricting the movements of the people to minimize the contact between man and fly. This reduced

\* Our thanks are due to Dr E. D. PRIDIE, C.M.G., D.S.O., Director of the Sudan Medical Service for permission to publish this paper. Also to Mr D. J. LEWIS M.A., Medical Entomologist to the Sudan Government, and to Dr R. KIRK, Government Bacteriologist, for much valuable help and advice.

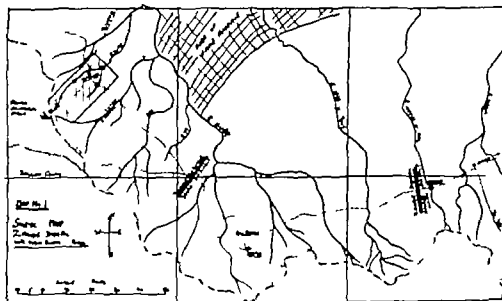
and controlled the disease, but necessarily imposed many irksome restrictions on their tribal habits and customs. The danger always remained that a serious outbreak would occur if these restrictions were relaxed.

In 1937 Mr C. B. SUTHER, Medical Entomologist to the Kenya Government and the originator of the "Block" system of control of *Glossina palpalis* ssp. *fuscipes* visited the area. The present experiment was started in the following year.

The main object of this paper has been to assess the value of this method of control as compared with the past measures adopted in the Sudan.

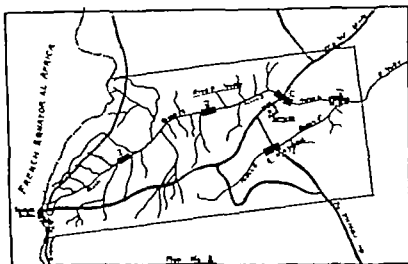
## II—THE DISTRICT AND THE PEOPLE

The area concerned is the Temburu sub-district which lies in the south-west corner of the Anglo-Egyptian Sudan on the Nile Congo divide. The two maps show the topography of the district and the area of the experiment in necessary detail.



The country on either side of the divide consists of low rolling hills with plenty of open savannah forest. Along the divide itself rise many small rivers and streams. All of these eventually drain into the river Sobat which enters the Bahr el Ghazal tributary of the White Nile. Nearly all these rivers and streams are infested with *Glossina palpalis*.

Table I shows the average rainfall, and temperature monthly for the last 5 years (the period of the experiment).



RIVER YUBU AREA.

Scale 1 inch = 10 miles (approx)

Sources Yubu the sleeping sickness settlement founded in 1920 is situated  $5^{\circ} 1' 10''$  N and  $27^{\circ} 26' 37''$  E and is 2,345 feet above sea level. The river Yubu rising here, runs north eastwards crossing the main road to Wau, 2 miles north of Tembura. It is later joined by a large tributary the Mayuku,

TABLE I

AVERAGE TEMPERATURES AND RAINFALL SOURCES, YUBU 1938-42.

	Maximum Temperature in C.	Minimum Temperature in C.	Rainfall in mm.
January	34.6	16	17.7
February	37.5	17	31.7
March	35.7	16	53.3
April	34.5	17.5	147.3
May	33.6	17.5	181.2
June	31.6	18	170.3
July	30.3	17.5	150.2
August	31.9	17	200.6
September	31.5	17.3	242.4
October	33.5	17	164.4
November	35.1	16.3	131.4
December	35.2	16.2	10.3

and eventually drains into the river Such. The tsetse fly control scheme covered the area from the source of the Yubu to its junction with the Mayuku and the whole of the latter tributary. The total area under fly control was about 300 square miles with a population of about 15,000.

The course of the river Yubu is similar to that of the other river systems. It rises as a series of small springs amid dense gallery forest, and winds about considerably. Most of the river in Block 1 is gallery forest. In Block 2 the gallery forest becomes more patchy and the bed of the river is wider as many tributaries join it. In Block 3 the river bed becomes more definite and the forest country more open, with an occasional patch of gallery forest. In Block 4 the river banks are clean cut, some 5 to 10 feet deep and there is little or no gallery forest. The type of riverain vegetation here is probably the most suitable for *G. palpalis*.

The stretch of country running along the frontier is the most densely populated area of the district, as it is the most fertile. The people belong to the Zande Tribe. EVANS PRITCHARD SELIGMAN and others have described them in various anthropological books and papers. They are agrarian, short, stocky and well built. They were mainly forest dwellers but the advent of sleeping sickness to their domain forced the Government to alter their mode of existence when the epidemic was at its height. Instead of living in small family groups isolated in the forests, they were moved along roads and forced to use specified watering places. This facilitated the control of sleeping sickness, and effectively reduced it, but for the people themselves created many irksome restrictions.

The importance of sleeping sickness in the area is firstly to control it so that no major epidemics occur and then to prevent its spread to other parts of the southern Sudan where it is not yet endemic. Any scheme which will eliminate or reduce the disease to negligible proportions will increase the economic possibilities of the country and the welfare of its peoples.

### III—THE DISEASE. BRIEF HISTORICAL SURVEY

The possibility that the disease might be endemic in the southern Sudan was realized as early as 1905 and a commission was sent to investigate. At that time the area under consideration was little known and administered. No cases were then found, though the incidence of *G. palpalis* was recorded (ENSON, 1908). MAURICE's accounts of the history of the disease in the Sudan (MAURICE, 1930) are worthy of special study and need not be elaborated here.

The infection was undoubtedly introduced about 1916-17 to the Tembura sub-district from French Equatoria. In 1918 255 cases were found. These were segregated near Tembura. In the succeeding years the district was opened up and many more cases were found. This resulted in the formation of the sleeping sickness settlement at Sources Yubu in 1920. In this settlement all cases were segregated with their families. The incidence of new cases continued to rise in an alarming manner and in 1923 over 800 new cases were found in the Tembura sub-district. There was little doubt that many more existed, and the staff was increased to cope with the problem. Sleeping sick

ness regulations were introduced and the following is a summary of what was done to control the disease —

(a) New roads were cut, along the watersheds as far as possible so that streams were avoided or crossed at right angles. The population was then forced to live along these roads and the roads divided up among the various chiefs and sub-chiefs. Protective clearings, maintained by the people were made at road river crossings and at all watering places.

(b) All cultivations and houses had to be within sight of these roads

(c) A census of the population was carried out. Inspections were done monthly of the whole population.

(d) Transfrontier traffic and traffic through the infected area was restricted

(e) Hand in hand with the medical work the native administrative authorities (chiefs and sub-chiefs) were encouraged and from the first they were expected to enforce the regulations.

These drastic regulations did control the disease and in 1929 there were only eighteen new cases

Seven years later the incidence of the disease again increased. This was due to the following factors. The disease was no longer so real a menace to the people, and the regulations imposed were being less and less strictly observed. The area along the roads were overcropped and cultivations were being made further and further away from the roads. Alongside the cultivations were new houses. Clearings were neglected and inspections poorly attended.

In 1937 the regulations were amended a new and accurate census was prepared in conjunction with a card index system for the whole population. The frequency of inspections was reduced to 3 monthly but they were more thorough. Absentees from inspections from this date onwards have almost invariably been under 4 per cent., and the majority of these are traced within 2 months. Bayer 205 had in the meantime replaced atoxyl in the routine treatment of new cases. As this drug rapidly sterilized the peripheral blood of trypanosomes the isolation of patients in a fly free settlement was discontinued. The size of protective clearings was increased to 200 yards square. These measures did not reduce the incidence of the disease as was hoped.

At the time of Mr STILES's visit the Yubu river system was still providing a large number of cases and was the most dangerous area. For this reason the experiment in fly control was started here.

#### IV—THE FLY LOCAL SPECIES AND DISTRIBUTION

##### (a) LOCAL SPECIES

Fly catches in the area covered by the block scheme show that over 98 per cent of all *Glossina* caught were *G. palpalis* ssp *fuscipes*

Mr C. B. STILES in his report on the possibility of initiating a block scheme made the following remarks on the species of fly found in the area. This report is a Government document that has not been published.

"Species associated with human trypanosomiasis



*G. submorsitans* appears not to be connected with human infection. It occurs with *palpalis* on one area on the Bakiri road on which slight infection occurred during 1937 but as far as it was possible to ascertain in none of the heavily infected areas. Its distribution perhaps, overlaps that of *G. palpalis* on the lower portions of the Yubu and Such rivers, and probably on other rivers to the north. It might possibly have been concerned with human infection in the past, but it is apparently not guilty at present. The *morsitans* problem is therefore, I consider quite distinct from that of *G. palpalis* and sleeping sickness. Surveys and studies will be required to discover possible methods of elimination.

*G. fuscipennis* has not yet been incriminated in the transmission of human trypanosomiasis, and little is known of its habits."

It is considered then that—

"*G. palpalis* sp. *fuscipes* is the only tsetse fly concerned with human sleeping sickness in this district, and it is therefore the only species that need be dealt with in any measures designed for the control of the disease."

*G. morsitans* exists in the area, though not in any great numbers. Several interesting factors concerning *G. morsitans* have been noticed by various observers who have worked here on sleeping sickness. In 1908 ENSOR noted the large numbers of this fly all the way from Tembura to Bo, a river post some 60 miles south of Wau. MAURICE noted that when he first went to the district, and before the people were moved on to the roads that most of his mules and ponies quickly succumbed to trypanosomiasis. After the people moved on to the roads, *morsitans* became more common and mules and ponies went out of use. MAURICE (1930), however records that after this move *G. morsitans* seemed to leave the roads entirely.

The authors have noticed that in this country (and this includes the whole of Zande district as covered by Map 1) *G. morsitans* follows game rather than humans. The Azande are persistent hunters. The game soon leave any locality in which they settle. Thus the very advent of people to an area drives away or appears to drive *G. morsitans* with the game.

These points are mentioned because ARCHIBALD reported two cases of *T. rhodesiense* from this area in 1926. The possibility of a change from one form of trypanosome to another with a change in the fly vector is quoted by BOURGIGNON (1938) and others. This might account for ARCHIBALD's two cases.

*G. palpalis* is by far the most common fly caught in the area and there can remain little doubt that this fly is the responsible vector.

#### (b) SOURCES OF FOOD FOR FLY

The commoner animals that might be food supplies for the tsetse are bushbuck, pig, waterbuck, birds various lizards and other reptiles. STANES, in

his report mentions the following results of precipitation tests which he carried out —

Out of 625 flies caught 232 contained blood in small amounts.

Thirty-one (5 per cent.) were positive to human sera and two to waterbuck. The positive reactions to human antiserum may mean monkey or human blood.

Out of 100 selected specimens taken from the upper Yubu seventy two contained blood. But none of these were positive to human, waterbuck or pig antisera."

STANES also notes the high percentage of females caught. This he regards as evidence of the hunger of the fly. This has been borne out by our catches in the block scheme (see Table II)

TABLE II  
FLY CATCHES IN SOURCES YUBU BLOCK SCHEME.

Year	Total Male	Total Female	Grand Female.	Per cent. Females of Total Catches.
1940	35 074	69 148	15,201	66.4
1941	51 058	71 485	29 985	58.6
1942	34 434	38,492	16,888	52.7

STANES's figures were from 52 per cent. to 60 per cent. females of the total catches

### (c) PUPAE.

Intensive searches for pupae have been made on several occasions but only a few were found. The difficulty of finding them in riverside country has similarly been recorded in Uganda (McCONNELL, 1912 and GIBBINS 1941) in Kenya (STANES and VANE, 1937), and in Nigeria (JOHNSON and LLOYD 1923)

### (d) GENERAL NOTES ON DISTRIBUTION AND BEHAVIOUR OF FLY

In the main, *G. palpalis* behaves as has been recorded by other observers. GIBBINS (1941) records that *G. palpalis* is always within 10 yards of the water edge unless carried further away by its host. This we have not found to be invariably the case. It must be remembered that in the Sudan the northern limit of *G. palpalis* is reached. It is possible that near this limit its behaviour differs somewhat from that under other conditions further south. BEDFORD (1930) places the limit of *G. palpalis* in the Sudan as 9° N. and on a line running parallel with the southern boundary of the Anglo-Egyptian Sudan, and roughly 100-150 miles north of the boundary

### V—THE DEVELOPMENT OF THE FLY CONTROL SCHEME.

The scheme applied to the river Yubu area was materially the same as that described by STANES (1935) and by STANES and VANE (1937). Geographical

differences and a lower fly incidence allowed certain variations. In Kenya, STRAIN recorded an original fly density of 168 per fly boy day whereas in the Yubu river system the highest density recorded was 16.1 per fly boy day with an average of nine to twelve per fly boy day at the beginning. Our blocks were therefore made considerably longer.

In 1938 fly catching was started in Block 1. By January 1939 the river as far as its crossings with the Wau road just north of Tembura was under fly control. This was divided into three blocks. In 1940 Blocks 4 and 6 were opened and finally Block 5 was opened in 1941.

The scheme had to be developed slowly so as to include the whole of the area. The river itself had to be mapped and surveyed and all tributaries listed and recorded. As one section was brought under control another was prepared for control. The area north of the Mayuku Yubu junction was left. It was uninhabited and not much frequented by the people.

Fly boys paths were made on both sides of the main river and all its tributaries and as close as possible to the river banks. In all about 300 miles of fly boys paths were cut.

The barrier clearings were all made 800 yards long and 400 yards wide, being 200 yards wide on either side of the river. Where a tributary entered the main stream a Y-shaped clearing had to be made, and here it was cleared for 400 yards along each limb of the rivers.

The clearings were limited to five largely for reasons of economy. The actual siting of them was also affected by the same motive, and Clearings III and V were both at road river crossings where previous small protective clearings had been made. This was not altogether satisfactory as the traffic across the roads attracted fly and they were not absolute barriers. Clearing III was eventually enlarged until it was 1 000 yards long but even then a few flies were caught at the bridge in the centre. However the flies caught in the clearings were so few that it was obvious that the clearings acted as fairly effective barriers against fly movement.

The maintenance of these clearings was a difficult problem. The rapid growth of the local spear grass necessitated clearing three or more times a year. Various cover crops were tried out and the most successful of these to date has been colopogonium. As the scheme progressed, and the disease incidence fell, it was decided to cease clearing the numerous small road-river crossing clearings, and the watering place clearings covered by the area of the scheme. Instead of doing these, the people were asked to maintain the barrier clearings as a civic duty. The work entailed was less than a fifth of that which they had to do before.

The most important clearing was Clearing IV as north of this there was no fly catching. In 1943 the maintenance of low fly catches enabled us to cease the clearing of all barrier clearings except this one, so the work of the



FIG. 1.—Source Yuba. Fly Boys along the River Yuba.

FIG. 2.—River Yuba. Barrier Clearing No. 4 after initial clearing, showing the amount of debris to be removed before clearing can be completed.



FIG. 3.—Barrier Clearing No. 3

FIG. 4.—Edge of Barrier Clearing No. 3, showing experimental corn crop of Colopogonium.

differences and a lower fly incidence allowed certain variations. In Kenya, STRAUS recorded an original fly density of 168 per fly boy day whereas in the Yubu river system the highest density recorded was 161 per fly boy day with an average of nine to twelve per fly boy day at the beginning. Our blocks were therefore made considerably longer.

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The most important clearing was Clearing IV as north of this there was no fly catching. In 1943 the maintenance of low fly catches enabled us to cease the clearing of all barrier clearings except this one so the work of the

people was reduced even further and the efficiency of this clearing was increased by continual clearing instead of periodic clearing.

The fly catching was done by small boys aged from 11 to 16 years. They wore dark blue uniforms and were equipped with small hand nets. Each boy also carried a cane knife or hoe for maintaining the footpaths. In each block there was a "group" of boys under a group leader. The groups were subdivided into sections under a section leader. Each section had a set portion of the river to patrol. The number of boys in the section varied according to the length of the river in the section and the fly catches obtained. It was found that it was best if the boys caught as a team rather than singly or in pairs distributed over the whole of their section. The boys lived in specially made camps near their work. When the scheme was first started there were only thirty-eight boys. During the last 5 years the number has increased and the total staff employed now consists of six group leaders, twenty-six section leaders and 220 fly boys.

In addition there are three trained overseers who supervise the work of the groups in the various blocks.

At the end of each day the flies caught are collected by the section leaders and each boy's catches are recorded. The flies are divided into male, gravid female and non-gravid female. Typical specimens are kept for identification and if necessary are sent to the Medical Entomologist. The group leaders again check these flies, and at the end of the month they are finally checked and counted by the Medical Officer at Sources Yubu.

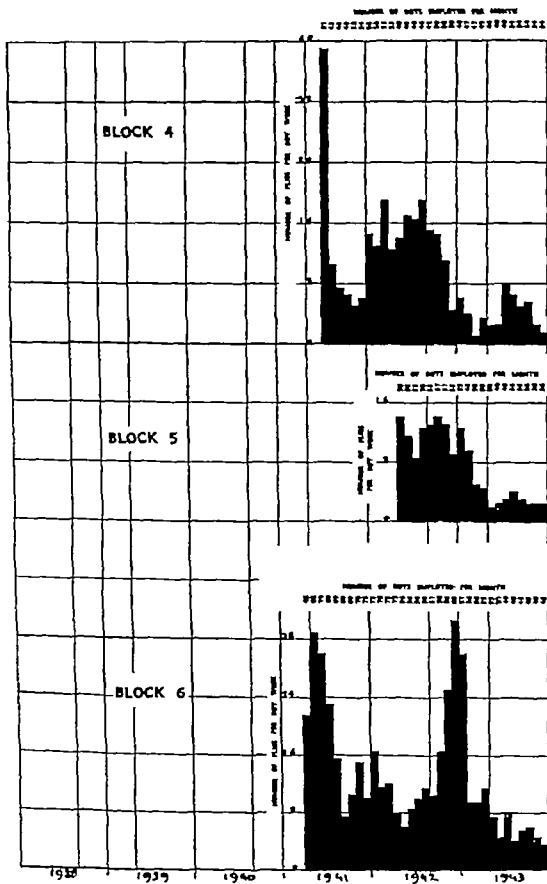
Until the end of 1941 a large part of the funds available was spent on making new barrier clearings, maintaining the old ones and opening up the new blocks. As the people themselves took over most of this work more money was available for the fly control staff. This enabled us to employ more fly boys and increase the efficiency of the scheme.

## VI.—RESULT OF THE SCHEME ON THE FLY

The effect of control measures is shown in the graph. The fly catches in the area of the scheme are shown, monthly, for the last 5 years. These catches are expressed as flies caught per boy per week. The graph also illustrates the growth and gradual development of the scheme over the whole area.

This method of expressing fly catches per week instead of per hour has not been used previously by entomologists and possibly needs some explanation. The whole-time entomologist, with trained and reliable personnel under his supervision, can give full supervision to fly catching. With our organization it would have been impossible to obtain figures per fly boy hour that would have been reliable for the whole area. Originally fly catches were expressed as so many flies per fly boy hour, but then later as the catches became less and less they were expressed as flies per fly boy week.





FLY CATCHERS RIVER YUKU AREA, 1938-1943. Catches expressed per fly boy week.

Note.—The graph shows the development of the fly-control scheme during the period and the number of boys employed in each block.



The method has its advantages. For example, catches expressed per fly boy hour are usually sample catches taken at varying intervals and at special localities, for a short period during one day. Our catches are taken over the whole area for continual work. They thus indicate better the risk run by a native using that area for hunting and so forth.

Experiments were carried out to obtain comparative figures of these two methods of expressing the fly catches. Accurately timed catches were made in one section over a period of 2 weeks. The results were that catches of one per fly boy hour were found to be the equivalent of thirty per fly boy week.

A study of the graph shows that in all the blocks a reduction in the fly catches was obtained quickly. In all blocks within 6 months the fly catches had dropped to very low levels.

The chief problems we have found is the maintenance of these low fly densities. The difficulty is to decide what is a safe low density and the minimum amount of work necessary to maintain it. TEESDALE (1940) found the fly could not be eliminated completely by fly catching methods. It was originally suggested that once densities were reduced to a very low level the remainder of the fly would die out. This was not so. The authors findings have coincided with those of TEESDALE.

Seasonal variations in the numbers of fly caught are to be expected. In the rainy season the catches tend to rise in spite of the fact that the boys are not at work while it is raining and thus several days work may be lost per month. No definite variations in the numbers of gravid female non-gravid female or male flies caught were noted during the various seasons.

In Block 2 there was the greatest difficulty in maintaining fly catches at a low level. But in two sections of this block additional streams which had been dry in later years had water in them. These were then cleared right up to their sources and extra staff employed.

Block 2 is approximately 12 miles long, and contains some 100 miles of stream. It is the largest block in the whole scheme. Undoubtedly we did not staff it with sufficient fly boys originally and the overseers failed to notice certain heavily infested pockets of fly country.

From May to December 1942 Block 1 was left without any fly catching at all. The catches in this block had never been high, and had been maintained at less than five per fly boy week for over 3 years. Fly catching for 14 days was done in July and December 1942, to see if there had been any appreciable increase in the fly. It was found to be three per fly boy week in July and less than one in December. Of necessity there must be a time lag before there is any noticeable increase. Catches in 1943 showed that by April there was an increase to over five per fly boy week. Sections of fly boys soon reduced this and in the succeeding 6 months catches were maintained round about two and a half per fly boy week.

Graphs are kept of the catches in each section of each block per month.

If any section shows an increase the cause is investigated and if necessary more boys are posted to that section.

Thus any part of the block scheme area can be checked and controlled easily.

We have tried to maintain an even "fly density" of five or less per fly boy week. This may seem ridiculously low to some observers and although it may be considered an ideal it had been attained over most of the scheme by 1943 and kept at that figure.

To summarize the effect of the scheme on the fly it can be said that over the whole area of the scheme the fly catches show a drastic reduction in the numbers of fly caught. These low catches have been maintained. There is evidence that once low densities have been attained and maintained for a long period it takes a long time before the fly increase again. Complete elimination of the fly is not possible but effective reduction and control can be and has been achieved.

#### VII.—THE EFFECT OF THE SCHEME ON THE DISEASE.

The incidence of sleeping sickness throughout the district since 1918 has decreased, especially after 1923 when the population was moved on to the roads.

In 1923 over 300 out of 839 cases detected were infected from the River Yubu. It will be realized that there is often considerable difficulty in obtaining case histories. The Azande are as suspicious of cross examination and as liable to lie as any other primitive African native. Movements across the frontier and unauthorized change of domicile are concealed if possible, from fear of punishment. In deciding the origin of infection unless there is definite evidence to the contrary cases are recorded as having been infected at the nearest stream to their homes or cultivations. It will be noticed that the incidence continued to decline after the population in this area were permitted to move their homes away from the roadsides (1940). This is evidence of the effectiveness of the scheme.

The distribution of cases from 1938-42 within the area of the block scheme is illustrated in Table III. It will be noticed that in 1942 there were only three cases and these came from one block (Block 2) where a pocket of fly infested country was discovered.

The highest number of cases traced to the Yubu River area in any one year was 300 (1923) and the lowest number was three (1942).

From 1928 to 1938 the incidence in this area fluctuated considerably but statistics show that 34 per cent. of all cases annually came from the Yubu River system. Infections from the other two river systems (Mongu and Biki) also maintained a steady proportion one to the other. By the end of 1942 although fly control had only been in operation for a period ranging from 5 years (in Block 1) to 2 years (in Block 5) the incidence of cases in the Yubu River system

TABLE III

SLEEPING SICKNESS CASES TRACED TO YUBU RIVER AREA COMPARED WITH CASES FROM OTHER PARTS OF THE TEMBURA SUB-DISTRICT FROM 1938 TO 1942.

Year	Yuba River Area (by Blocks)						Other Areas.	Remarks.
	1	2	4	5	6	Total.		
1938	14	81 cases from this part of Yuba Valley				85	28	Block system introduced. Block I opened.
1939	8	20*	25	3 cases from this part of Yuba Valley		54	45	Blocks II and III opened
1940	4	18	7	1	0	10*	37	41
								Blocks IV and VI opened. People allowed to move off the roads. (See text.)
1941	6	8	1	1	2*	0	19	57
								Pocket of fly country not previously included, and added to Block I
1942	0	0	3	0	0	0	3	41
								Similar pocket of fly infested country found in Block III

Fly catching started in this block.

had dropped to 7 per cent. of the total for the Temburu sub-district. This was a real reduction and not due to new outbreaks on the Mongu and Biki rivers.

It cannot be said that there has been any dramatic reduction in the number of cases from the area since the introduction of fly control, but the steady reduction in spite of the freedom of movement and domicile allowed to the people is evidence of the value of this method of fly control in an endemic area.

Sporadic cases are always liable to occur but in view of the reduction of fly population over the Yubu River system as a whole, the possibility of the disease ever again reaching epidemic proportion is negligible.

#### VIII.—DISCUSSION ON THE VALUE OF THE EXPERIMENT

Control of *G. palpalis* by application of the Block system has now been in operation over a comparatively small area for the last 5 years. During that period the following results have been achieved —

(a) The density of *G. palpalis* has been reduced. The continued low catches are evidence that there are fewer fly

(b) The incidence of sleeping sickness has been gradually but steadily reduced from sixty five cases in 1938 to three cases in 1942. The authors and others interested in the experiment have been amazed that with such low fly catches, and therefore presumably low fly densities, the disease still exists at all

GIBBINS (1941) quoted fly catching figures of two per fly boy hour as "safe." A comparison would mean that figures of sixty per fly boy week would be "safe." But he did not correlate his fly catching figures with the disease incidence. His figures were taken in specific localities at varying intervals. In this experiment the figures have been recorded daily for over 5 years over the whole area.

There is a definite period between the time of infection and the development of the disease to the stage when it can be diagnosed. Therefore one would expect a time lag between the reduction of the disease and the reduction of the fly.

(c) The disease has been controlled in spite of the fact that many of the irksome restrictions, previously considered necessary, were removed.

In 1936 and 1937 sleeping sickness was a serious problem in this small thickly populated area. By 1940 (after the experiment had been in operation for 3 years) the disease incidence had been reduced and the restrictions on where the people lived were removed. By 1942 there was a still further decrease in the disease, only two cases certainly having been infected within the area in that year. The other case, only a "possible," is included in the records.

Until 1940 the people had to live along the roads and draw water from certain specified places. The area available for their cultivations was not merely limited but was becoming over cropped. The controlled watering places had all to be cleaned every 3 months by the people themselves as a civic duty. The authors' confidence in the effectiveness of the scheme allowed all these restrictions to be removed. Thus an important area of country was opened up to the people for settlement and cultivation.

The maintenance of the fly control scheme even with reduced staff under war conditions has not merely controlled the disease, but a further decrease has been attained.

(d) The experiment has provided valuable information concerning the practicable possibilities of tsetse fly control in the Anglo-Egyptian Sudan.

(e) Sleeping sickness is also endemic in the adjacent Oubangui Shari Province of French Equatorial Africa. Cross frontier traffic with the natives of the other side is impossible to stop and difficult to control effectively as the people are all Azande. The fly control area is now a barrier to the disease. Any cases coming over from the French side are less likely to start a nidus of infection in the area and the reduction of the disease in the area on our side of the frontier makes it most unlikely that any cases will go to the French side.

#### IX—SUMMARY

(1) A small area where sleeping sickness was endemic in Equatoria Province of the Anglo-Egyptian Sudan was selected for experimental trials of the Symes Block method of tsetse fly control. The area is described.

(2) The disease was first recorded in this district in 1918. Since that date various methods of controlling it have been tried and these are described.

- (3) The application of the Block method of tsetse fly control is detailed together with the results of the experiment on the fly and the disease incidence.
- (4) The effectiveness of the experiment in tsetse fly control is discussed with particular reference to its advantages over the methods previously used in this area.

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## THE MERIDI OUTBREAK OF SLEEPING SICKNESS.

BY

J. F. E. BLOSS M.R.C.S. L.R.C.P., D.T.M. & H.\*  
*Senior Medical Inspector Sudan Medical Service*

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### I—INTRODUCTION

In February 1941 an epidemic of sleeping sickness broke out near Meridi in Equatoria Province of the Anglo-Egyptian Sudan. The area around Meridi had always been regarded as a sleeping sickness area (that is an area in which the disease might become endemic or epidemic) but no cases had ever before been traced to the district. The only case that had been recorded was an imported case in 1908.

This epidemic was very localized, and although only a few cases occurred it was quite severe considering the numbers of the population. The origin of the infection is not known but illegitimate transfrontier traffic with the Belgian Congo is suspected. This outbreak is of especial interest for the following reasons—

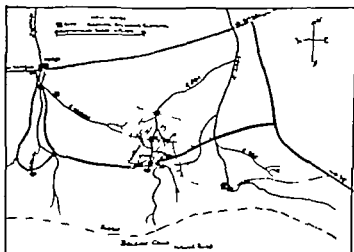
- (a) Its localized nature, and comparative severity
- (b) The experiments in tsetse fly control and their effect on controlling the epidemic.
- (c) The data obtained concerning the epidemiology of its spread within a small area.

\* Thanks are due to Dr. E. D. PRIDIE, C.M.O., D.S.O., Director of the Sudan Medical Service for permission to publish this paper. Also to Mr. D. J. LEWIS, M.A. Medical Entomologist to the Sudan Government to Dr. R. KIRK, Government Bacteriologist and Dr. A. R. HUNT for much valuable help and advice.

## II.—THE DISTRICT AND ITS PEOPLE.

The area concerned lies roughly inside the square bounded by the following lines of longitude and latitude  $29^{\circ} 30' \text{ E.}$  to  $29^{\circ} 45' \text{ E.}$  and  $4^{\circ} 45' \text{ N.}$  to  $5^{\circ} 0' \text{ N.}$  The Map shows the relevant topography of the country. This map is not to scale, but it shows all that is necessary and is easy to read.

The first cases were all found at the spot where the river Eidi crosses the road (A). A rest house, the chief's house, and a big chief's court were centred here. This chief was a particularly go-ahead man, being the first chief in the province to build himself a brick house. A population density map, made as soon as possible after the outbreak had been discovered, showed that approximately 2,000 people lived in an area of 8 square miles around the place marked (A). People were always moving in and out of this centre and it was the worst place imaginable for a nidus of infection to be found.



SKETCH MAP OF AREA OF MENDE SLEEPING SICKNESS OUTBREAK

Towards the end of 1940 the people finding this area too crowded had asked permission to move and make their houses along a new road running south-east to the river Naam. This had been approved, and before the outbreak was discovered, several had already done so. This is of interest in studying the spread of the epidemic.

The people were a mixture of tribes—Baka, Mondu, Avokaia, with a few Azande. Most of them spoke two or more tribal languages as well as the bastard arabic of the south, and the common border dialect of Bangala. People of all the tribes concerned could be found in any one chiefship or sub-chiefship. These facts complicated the native administration, and in turn the application of sleeping sickness regulations.

The country along this section of the Nile Congo Divide consisted of low rolling hills, with many streams and much dense gallery forest. It differed

from that further west in that it had more granite outcrops and the depression type of gallery forest was common.

The river Eidi was a typical example of the rivers of the area. It started as a small spring on the border winding about between hills and with dense forest along its course. It collected many small tributaries and by the time it reached the chief's house (A) it was about 15 to 20 yards across. North of this the dense gallery forest was gradually replaced by more open savannah forest until about 6 miles north of (A) all gallery forest had disappeared. The river then became very tortuous with many small cataracts, and rocky pools and finally entered the Naam. Much of its course provided ideal breeding grounds for species of *Simulium*.

The river and its environs were much used by the people for hunting fishing and, in the dry season, for the eternal search for forest foods such as honey roots and herbs. Only a few people lived actually on its banks. South of (A) there were practically no people at all. The cultivations along it were patchy. There was remarkably little game along the river. A few buffalo waterbuck, bushbuck, pig occasionally smaller gazelle and duiker and monkeys including colobus could be found. Lizards and reptiles were common.

*G. palpalis* was common and frequent along the upper course of the river particularly around the chief's house (A). Here, a small clearing where the road crossed the river served no more useful purpose than to make the road obvious. In such small clearings tsetse were common. As the forest became more open to the north, *G. morsitans* was the predominant fly. In Block II of the fly control scheme, most of the catches were *G. morsitans* so fly catching was stopped here within 9 months of it being started.

*G. palpalis* could not be said to be present in large numbers anywhere along the river which was surprising. *G. morsitans* was most common in the areas where the game was to be found, and appeared to vary its habitat with the movements of the game.

### III—THE EPIDEMIC IN OUTLINE.

Prior to 1941 sleeping sickness inspections had been carried out all over the Meridi district once a year. These inspections had not been as thorough as in the Temburu area to the west, where the disease had been endemic for some years, but approximately 60 per cent. of the people were seen. This was considered a sufficient safeguard against a serious epidemic and in this instance it proved that it was.

At the end of 1940 the Sudan had been invaded and the war situation in the country was very serious. The army and emergency war organizations needed all the doctors available. In the southern Sudan, and especially in the out stations staffs were reduced to a minimum. Not only was staff short, but changes were frequent and the inspection of this area which should have been held in August, was postponed. Having due regard to the past history



of sleeping sickness in Meridi, no one could say this was dangerous. The inspection was held in February 1941 some 60 per cent. of the people were seen, and seven cases of sleeping sickness were found (*T. gambiense*).

At such a time the danger of the outbreak was serious. To the west was a part of Zande district where the disease had never broken out. To the east was Amadi and Moru district with rivers ideal for and full of, *G. palpalis* and again the disease had not occurred here. To the south lay Yeti district where the disease had once been seriously epidemic, but now was all but non-existent. This area was later to prove of military importance in the African lines of communication. There was much inter tribal traffic in all directions and this would be impossible to stop and difficult to control effectively. Those who had moved from the central area (A) had gone to new places and might start localized outbreaks elsewhere.

The river Eidi had never been surveyed, and was inaccurately mapped. A rough survey of this river its course, tributaries, and the distribution of the people along it were noted, and the necessary maps prepared.

The catchment area of the river was found to be very compact. Its water shed ran along low hills roughly 3 miles east of it. Very few families lived to the south of the chief's house. The population lived immediately to the north of (A) and along the river and the large tributaries which entered it near this spot.

The outbreak was found to be very localized, all the first cases being resident in the area immediately around (A). The following were the measures taken —

(a) A block system of control for *G. palpalis* was started. This was based on the work done at Sources Yubu (HUNT and BLOOM, 1945). The first barrier clearing was made at (A). The second and third were made 8 and 11 miles downstream respectively dividing that part of the river into two blocks. Outside this area no cases had been found. The whole river with its tributaries inside this area was brought under fly control. Inside this control area no people were allowed to live or to have cultivations except along the roads. Traffic through the fly control area was forbidden except along new roads, and no hunting or forest excursions allowed. Two new roads running north, and on either side of the river marked out the boundaries of the fly control area. Along the main road inside the control area people had to have their houses in sight of the road and were allowed to draw water only from certain specified watering places. Outside the fly control area no restrictions were placed on the people at all. Watering places on the new side roads were fortunately so placed that they did not connect with the Eidi or its tributaries. Thus fly from the Eidi were not likely to migrate into the new river systems being used.

(b) All householders were given numbered discs and an accurate census of the people was made. This census was recorded on cards, one card for each householder the names of those under his charge being recorded.

(c) Inspections were held 3-monthly of all persons.

Staff experienced in fly control work were brought over from Sources Yubu to start the scheme locally as there was no sleeping sickness staff at Meridi. Unfortunately the person in charge of this, a Sudanese Public Health officer contracted cerebral malaria within 2 months of arriving and had to be invalided home. He was not replaced at once, so both Sources Yubu and Meridi lost

but locally trained southern staff carried on and the fly control scheme was in full working order about the middle of May.

It was not possible to extend fly control to areas other than at (A). The danger of an outbreak on the river Naam was realized but it was hoped that frequent and thorough inspections would reduce the risk there.

The labour problem alone was difficult. The cultivation season was at hand. To interfere with that would probably create a food shortage in 1942. Many people had moved and they had to clear the forest, build houses and sow their crops. It turned out that those who moved found excellent soil and had almost record crops—an event even the most optimistic could not foresee. Huge barrier clearings had to be made in areas of dense gallery forest. The photographs show the work of clearing that had to be done. One of the most difficult problems was to remove the tree stumps from the river bed.

By the end of 1941 a total of forty-seven cases had been found, all of whom had undoubtedly been infected on the Eidi at or near (A). Of these forty-three were first stage cases and four were second stage cases. This number represented about 2½ per cent. of those living in the neighbourhood of (A).

In 1942 work was at first continued on the same lines as in the previous year. In May it was feared that other rivers than the Eidi were sources of infection. Of these the Naam was the most likely and in July a localized form of *G. palpalis* control was started. This was a new experiment and is fully described later. Suffice it to say that fly catches here were reduced from 209 per fly boy week in July to under 10 per fly boy week in December.

This new road down to the Naam was the area which took most of the people who had moved from the overcrowded area round (A). The road river crossing at (B) was one of the best all the year round watering places in the district and fly catches there were the highest ever recorded in the Sudan.

Among the other rivers so involved were the Rasuba (D) and the Mbaraba (C). The Rasuba affected less than twenty people and probably was never a danger spot. The Mbaraba was an important tributary of the Meridi which ran into that river just 3 miles south of Meridi town. Two cases were found on the river though they probably had been infected on the Eidi. The possibility of infected fly getting to the Meridi and thence to Meridi town was considered a sufficient danger to warrant the starting of a small fly control scheme between the Mbaraba and the Meridi road river crossing. Fortunately no more cases were found on the Mbaraba and this scheme was dropped in 1943.

In 1942 there were twenty-five new cases, of which twenty-one were in the first stage of the disease. Of these cases four had probably been infected on the Naam.

In 1943 a further nine cases were found. Of these three were probably infected on the Eidi and six on the Naam.

This localized outbreak of sleeping sickness was thus brought under control within 2 years and the danger of its spread minimized by the control

measures taken. Among these the control of *G. palpalis* took an important place. A further study of the cases has also provided some interesting material concerning the epidemiology of the disease.

#### IV—THE CONTROL OF *G. palpalis* IN THE AREA.

There was no one settled method of fly control in all of the rivers. Various experiments were tried out in different places. These are described according to the locality in which they were carried out.

##### A. THE CENTRAL AREA AROUND THE CHIEF'S HOUSE.

A small block system of fly control was started here. This was based on SYMES' original work in Kenya with one or two minor modifications. The purpose of this control was

1. To reduce the fly in the infected area.

2. As this was the most thickly populated area to provide a fly-free and thus an infection-free area for the future. From previous experience it was thought that the reduction of fly density to a safe low level should be possible quickly and that all sources of infection (infected man as well as infected fly) should be eliminated within 2 or 3 years.

The first map shows the layout of the control scheme. This included about 12 to 15 miles of river with an additional 60 miles or so of tributaries. The barrier clearings were made somewhat smaller than those originally advocated by SYMES. Clearing I was 800 yards long and 150 yards wide on either side of the river. A small tributary the Nambirungwa entered the Eldi in the clearing and the upstream end of this clearing had to be much wider. The diagram shows this. Clearings II and III were 800 yards long by 300 yards wide, being 150 yards on either side of the main stream. These sizes were largely dictated by necessity. It was important to get the fly control scheme going quickly and labour was short. It was considered better to have smaller clearings which might prove effective barriers and to have some form of fly control working rather than delay the onset of the work. If these clearings were not effective barriers then they could always be enlarged at a later date. It is worth noting that a clearing has to be of a prodigious length before it is an absolute barrier to fly movement. Provided the clearing was a sufficient barrier to enable the fly to be reduced by hand-catching it was of some use.

Fly boy paths were cut along both sides of all tributaries, as well as along the river Eldi. Table I shows the fly catches in each of the blocks. By the end of 1941 no cases could be traced to the area of river covered by Block II and as most of the catches in that block were *G. moritani* fly control was stopped here. This block remained a closed area until the beginning of 1943 when it was opened up to the people, and sleeping sickness restrictions removed.

The diagram shows the area around the chief's house at (A). It will be seen that the river curves round from the chief's house to enter Clearing I. Nearby



FIG. 1.—River Elda. Site of Barrier No. 1 while clearing was being made.

FIG. 2.—River Elda. Site of Barrier Clearing No. 1 after clearing had been made.

FIG. 4.—Edge of Clearing on River Naam showing the Staggered Screens.

FIG. 3.—Tributary of River Elda entering it in Barrier Clearing No. 1. All the stumps still have to be removed.

FIG. 5.—Staggered Screens on the River Naam Clearing.

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The diagram shows the area around the chief's house at (A). It will be seen that the river curves round from the chief's house to enter Clearing I. Nearby

## B THE AREA ON THE RIVER NAAM

It was not until June, 1942, nearly 18 months after the outbreak had been discovered that the Naam at (B) was considered definitely to contain infected fly. When this was decided a trial fly catch was done and the incidence at the road river crossing was found to be over forty per fly boy hour. Within a minute of arriving at the water's edge I was attacked by three fly and bitten once. All the women washing clothes and drawing water by the bridge were continually being attacked. As soon as they saw we were catching fly they called to the fly boys to catch the fly off their backs.

There was no staff to initiate a block scheme of fly control so some form of localized control had to be effected. A central clearing 100 yards square was made and from this two rod shaped clearings each 200 yards long and 25 yards wide on each side of the river were made. Seven fly boys were stationed at fixed points in this rod protected clearing as it was called (Diagram 2 explains the layout.)

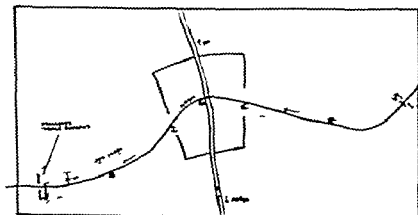


DIAGRAM 2

Rod protected clearing on R. Naam at Site (B) on Map p. 60

(Numbers refer to position of fly boys.)

In the first week the fly catches averaged 209 per fly boy week. This was the highest recorded fly catch in the Sudan. The boys stationed here catch on one side of the river one day and the other side the next. By December the catches in the clearing had been reduced to less than ten per fly boy week and the people said that the fly had gone.

In December a further experiment was carried out. GIBBINS (1941) by placing a cloth screen in the middle of one of his rod clearings proved fly travelled along the course of the stream rather than by taking short cuts across it. It was decided to experiment with similar screens but to place them at either end of the clearing in the hope that all fly would be stopped at the end. The result should be that the boys in the centre would catch no flies at all. Two screens, made out of bamboo and thatch, were put across the river extending

10 yards up either bank. Table II shows the catches before and after the screens.

After this it was felt that these screens at either end of the clearing would improve the efficiency of any clearing as a barrier to fly movement and screens were put up at either end of all clearings in the Meridi area. In order further to improve the efficiency of the screens two secondary screens were placed behind the first and further out from the river so that their inner edges overlapped the outer edges of the central screen. These were called staggered screens. It was thought that any fly more than 10 yards from the water's edge would be guided back to the river and attracted by the light and shade between the screens. A fly boy was stationed on the forest side of the central screens. (See Plate, fig. 2.)

TABLE II.  
FLY CATCHES, NAAM CLEARING BEFORE AND AFTER SCREENS.  
CATCHES EXpressed as flies per boy day

Boy No.	1	2	3	4	5	6	7
Before screens.							
Average of 3 days	9	* 3	4.7	4	2.3	3.3	4.3
After screens							
Average of 10 days	10	0.3	0.6	0.3	0.1	0.5	* 1

The fly catches along the River Naam at this clearing are shown in Table III.

The Naam at this point was without doubt a dangerous area. The people for a long way round used it as a watering place. The fly incidence was very high and cases of sleeping sickness had moved there before this disease was diagnosable. In June, 1942, cases were found which were almost certainly infected there, and one had to presume that the fly were infected. The fly control measures reduced the fly in this much frequented watering place and without doubt helped control the disease in this area. There was no fly catching in the river in territory at either end of the clearing and even when the fly incidence was high the fly incidence in the centre of the clearing was kept at low levels.

### C. THE VIBARABA MERIDI AREA.

Two cases of sleeping sickness were traced to the upper part of the Vibaraba. This important tributary enters its parent stream about 3 miles south of Meridi town. Both these cases had recently moved to this river without informing their chief and without the knowledge of the chief of that area. The possibility that infected fly might migrate downstream and enter the Meridi and thence get to Meridi town was considered of sufficient importance to justify some fly

TABLE III

FLY CATCHES, NAAMI CLEARING EXPRESSED AS *flies per boy week*.  
(For reference boys numbers see Diagram No. 2.)

	Boys Numbers						
	1	2	3	4	5	6	7
1942—August	81	34	20	28	35	64	94
September	51	16	9	16	22.5	28	26
October	31	12	8.5	13.5	13	22	18
November	33	14	5.5	6	11	8.5	16
December	30	4.3	7.6	8	3	6.3	16
1943—January	25	7.5		10.3	5		14.3
February	12.5	5.3		3.5	2.5		1*8
March	14.3	4.5		3.8	2		1*5
April	24	8.2		5	7.2		23*
May	37	12.2		11.2	9.2		22.5
June	57	5*	8.5	10.5	4	10.2	46.5
July	117	23.2	22.2	20	3	31.2	199.2
August	52.7	11.7	18.7	45*	22.5	14.2	63.7
September	33.7	16.5	13.2	14.5	12	20*	3*1
October	23.2	16.2	14.7	13.0	10	7.2	11.5
November	35.7	16.7	15.7	10.5	7	9.2	17*

control measure in this area. A barrier clearing with staggered screens was placed at the junction of the Mbaraba and Meridi and a narrow barrier clearing with screens was made on the Meridi where it crossed the main road. The block within these two clearings was patrolled by fly boys. Their catches were as follows (per fly boy week) —

1942—	May	June	July	Aug	Sept.	Oct.	Nov	Dec.
	4.5	6.7	25.2	18.3	20.3	10.7	9.6	13.2

Early in 1943 no further cases had been recorded from this river so fly control was stopped here. It is more than probable that the danger was not so great as was first feared.

The control of *G. palpalis* has now resolved itself into two main camps. The one favours a block system of control with continual fly catching. The other favours rod clearings often of great length, and controls these with periodic test catches. The rod clearing has never been considered as a barrier to fly movement, rather the reverse. By virtue of the fact that it is unsuitable for fly flies are said to move rapidly through it. The same, of course, may be said of a big barrier clearing. The disadvantages of a barrier clearing are its size (if it is to be a real 100 per cent. barrier) and the problem of maintaining it as a clearing. A long rod clearing is often more destructive of forest than a



barrier clearing for it must be remembered that the densest, most luxuriant, and most valuable timber lies closest to the stream. Tsetse fly control presents different problems in different localities and it is necessary to adapt certain methods according to the needs of that locality.

The rod protected clearing on the Naam with continual fly catching did in effect reduce considerably the number of fly in that one place. That was all it was intended to do. The value of the screens as a partial barrier to fly movement has been demonstrated, and the possibility of localized fly control in one special area proved.

The block system of fly control on the Eidi reduced fly there. Similar reduction on the Yubu has also been recorded (HUNT and BLOSS, 1945). In these instances fly control was needed over a wider area of river and a block system was the only answer.

Further experiments are being carried out to find if narrower clearings with screens could be a sufficient barrier to reduce the fly along any stretch of river and so have a place in a block system of fly control.

#### V — THE EPIDEMIOLOGY OF THE DISEASE

It is relevant to start this section with a brief note as to the methods used in the Sudan for the diagnosis and control of sleeping sickness. When the disease is endemic in any area inspections are held 3-monthly. At these inspections the cervical glands are palpated. Those with suspicious glands are gland punctured. If a case has a negative gland puncture but is highly suspicious, it is sent to hospital for further gland puncture and for a triple blood centrifuge. After the doctor and his trained palpators have finished, the native administration clerks call the roll from their census cards. A good inspection is one when over 95 per cent. of the people have been seen. The chiefs are given 1 month to find their absentees. The diagnosis thus rests on palpation of the glands of the neck. Up to 1 000 people may be seen in 1 day and the task of palpation, being largely negative, is not easy. The hot sun, oily sweaty skins, and the monotony of the work make it far from a pleasant job. Cases may be, and can be, missed but experience in the Sudan over the last 20 years shows that in spite of all difficulties these methods have kept the disease in check.

Where the disease is not known to exist, but where it might occur inspections are done once a year and no accurate census is kept. One hopes for over 80 per cent. attendances, and this should be sufficient to find out if the disease has occurred.

In the initial inspection on the Eidi 60 per cent. of the people were seen. Most of the absentees were women and children so that it would be fair to say that someone out of 90 per cent. of the households was seen. At this inspection seven cases of sleeping sickness were found.

Once an outbreak has been discovered the problem is to locate the river

system that contains infected fly and to control the persons living along that area. The fly-man contact must be broken. Fly control schemes take time to do this and the most speedy method of doing so is to move all persons on to roads and to enforce the use of special cleared watering places. The value of fly control is that it shortens the time these restrictions need be enforced.

Among the first cases found at the Eidi (A) were one person whose home was on the Rasuba (D) and one who had within the last 3 months moved to the Aze (B). The person from the Rasuba was a court policeman who spent most of his time at (A) and therefore was probably infected there. The second person was almost certainly infected at (A) as that part of the Naam and Aze had been uninhabited before.

In February 1942 (1 year after the outbreak was discovered) the mother of the case from the Rasuba was found to be infected. She had scarcely ever been to the Eidi as she was an old lady and if she was infected there it was pure chance. The fly incidence on the Rasuba was negligible, one fly only being found by three fly boys in a day and then under supervision of a doctor.

It was not until 15 months had elapsed from the outbreak that a case was found that was probably infected on the Naam. After 2 years only four cases had been found that were infected on the Naam. In the 3rd year six cases had been traced to this river. But for the fly control it is certain there would have been far more.

The problem was to find some relation between the fly incidence and the disease incidence, and to find the time factor required for the introduction of the disease to a virgin area before cases could occur.

On the Naam the fly incidence was very high yet it took over a year before cases could be found. Against this, one has to offset the fact that gland palpation at 3-monthly intervals does not always find the infection even with care.

In the Tembura area to the west a small isolated outbreak was found only after a probable case had been introduced 12 months previously. In this instance a woman was divorced from her husband and returned to her home. She was very ill, and those who saw her said she was undoubtedly suffering from advanced sleeping sickness. A year later four cases were found on this river.

It would thus appear that to find cases less than a year after the introduction of one infective case would be lucky. That is with our methods of control. With monthly inspections cases would certainly be found sooner but this is neither practicable nor necessary where the disease incidence is low.

The next problem is to correlate the fly incidence with the disease. On the Eidi the infection rate for man was high. The fly incidence was low—never over 30 per fly boy week. On the Naam the fly incidence was high yet cases were remarkably few and took over a year to find. Fly control measures soon reduced the numbers of fly, so further data re the infection rate in man with a high fly incidence are not available.

At Sources Yubu (HUNT and BLOSS, 1945) the same anomaly of infection in man with a low fly incidence has been recorded. In his original survey of the area at Yubu, SYMES noted the low incidence of fly and commented on this.

Table IV concerning thirty of the cases infected on the Eidi, shows that out of a total of eighty-one cases, thirty came from ten different houses. That is, that ten households provided over 30 per cent. of the cases. All these households were originally on the banks of the Eidi and close to the bridge at (A). The fly incidence being low the only explanation that bears consideration is that in that area *G. palpalis* did not migrate far from people and thus infected those using the same watering place. Apart from this one would have to think of some other biting fly as the vector and that would have to be a household rather than a riverain fly. *G. palpalis* does frequently follow its host far from water and, as has been mentioned before, was found in houses near to the coffee plantation. It is conceivable that once the fly found a good home in a house it would feed off all members in turn and so make the infection a household one. This household incidence of the disease has not been recorded before. Table IV shows the details of these thirty cases according to their households. In some cases there was an interval of over a year between the cases of a house being diagnosed as infected. This suggests that direct inoculation might have been made.

The points of practical value from these facts are —

(a) The disease can establish itself even where the fly incidence is low. Therefore fly control measures have to be very thorough and effective. Experience in the Sudan suggests a "safe" low incidence would be under five per fly boy week with continual daily catching.

(b) The incubation period of the disease (*T. gambiense*) is long. Cases are rarely found within 6 months of infection and it may be a year before the disease is recognizable. It must be remembered that the early symptoms often do not cause the native to ask the doctor's aid. He is far more liable to suspect magic and to consult his own magicians.

(c) The household incidence of the disease in this outbreak.

## VI—RESULTS OF TREATMENT

The treatment of sleeping sickness cases was as far as possible standardized.

Cases were differentiated into first stage, and second or third stage by Pandey's test.

First stage cases were treated with six intravenous injections of Bayer 205 (later replaced by its identical British counterpart, antypol). Each injection was 1 gramme and the injections were given at 3-day intervals. The patient was tested for any undue sensitivity to the drug by having minute doses before the first injection. No cases of such idiosyncrasy were discovered. This drug rapidly removes the trypanosomes from the peripheral blood and some of the first stage cases were treated in the local dispensary.

TABLE IV  
DETAILED INCIDENCE OF THE DISEASE IN TEN FAMILIES (THIRTY CASES).

	1	2	3	4	5	6	7	8	9
	Case No	Sex	Age.	Date detected	Stage of disease	Home	Watering place	Source of infection	General Remarks and Notes
1	20 35 44 66	M F.C. F M.C.	20 10 30 10	8.41 8.41 11.41 5.42	1 1 1 1	A A A A	A A A A	A A A A	Coffee Coffee Coffee Coffee (fly boy)
All these cases were infected in the coffee plantations									
2	16 38  6. 76	M.C. F  M F	12 30  25 40	5.41 8.41  5.42 2.43	1 1  2 2	A A  B B	A A  B B	A A  A (A) B	Suspicious for 9 months and was gland punctured three times before positive.
All these four lived on the Eadi but moved to the Naam early in 1941									
3	3 40 43	M F M.C.	25 35 1	8.41 10.41 10.41	1 1 1	A A A	A A A	A A A	Both cases died. No 43 relapsed quickly to second stage and died 2 months later
4	24 27 56  64	F M.C. M  F	60 12 55  30	5.41 11.41 5.42  5.42	2 1 2  1	A A C  C	A A C  C	A A A  A	Gland punctured four times before positive
5	48 68	F M.	30 30	2.42 5.42	1 1	A A	A A	A A	Relapse second stage 2.43
Also wife of 68—ran away before full particulars could be taken and was never traced.									
6	11 51	M F	30 40	5.41 2.42	1 1	D D	D D	A A	
7	8 53	M.C. M.	10 30	3.41 3.42	1 1	A A	A A	A A	
8	59 72 75 77	F F.C. F.C. F	35 8 5 30	5.42 11.42 2.43 5.43	1 1 1 1	B B B B	B B B B	B B B B	These four moved their home to the Naam early in 1941
9	71 74	M. F.C.	25 15	11.42 2.43	1 1	A A	A A	A A	House by the site of present barrier clearing No. 1
10	72 78	M. M	35 18	2.43 8.43	2 1	A A	A A	A A	

For letters reference A, B, C, D see Map and text.

Second and third stage cases are given three injections of Bayer 205 (or antypol) and then a course of from five to ten weekly injections of trypanamide. The dosage used for an adult was 3 grammes intravenously. All cases having trypanamide were treated in hospital.

Results at Yubu showed that first stage cases tended to relapse after a course of only six injections of Bayer 205. At Meridi the course was thus prolonged for first stage cases. The relapses of first stage cases are shown in Table V.

It will be seen that the relapses tend to occur some time after the completion of the course of treatment. The average time in the figures given is just under

TABLE V  
RELAPSES FROM FIRST TO SECOND STAGE.  
TWENTY THREE OUT OF SEVENTY-ONE FIRST STAGE CASES.

Case No.	Sex	Age	Date discovered	Date relapse	Period relapse	Initial course of treatment	Notes
1	M.	25	Feb., 1941	Feb., 1943	4	6 B 6 T	
3	M	25	Feb., 1941	Feb., 1943	24	6 B 6 T	
7	MLC	18	Feb., 1941	Feb., 1943	24	6 B	
10	M.	28	May 1941	Feb., 1943	1	3 B 6 T	
11	M.	30	May 1941	Feb., 1943	1	6 B 6 T	
15	M	18	May 1941	Feb., 1943	21	3 B 6 T	
17	F	30	May 1941	Feb. 1943	1	6 B 6 T	
19	F	?	May 1941	Feb., 1943	21	3 B 6 T	
22	F.C.	10	May 1941	Feb., 1943	21	3 B 6 T	
23	F	28	May 1941	Aug. 1943	27	3 B 3 T	Relapses to third stage.
27	M.	25	Aug., 1941	Feb. 1943	18	6 B	
30	M.	18	Aug. 1941	Feb., 1943	18	6 B	
31	F	30	Aug. 1941	Feb., 1943	18	6 B 3 T	
34	F	25	Aug. 1941	Feb. 1943	18	6 B	Blind 11.43, after being 6 6 BE Oct.
36	F	25	Aug., 1941	No. 1943	27	6 B	
43	MLC	1	Oct. 1941	May 1943	9	6 B	Died 11.43.
45	F	45	N v 1941	Nov. 1943	24	6 B	
48	F	30	Feb. 1941	Feb., 1943	24	6 B	
51	F	40	Feb., 1941	Feb., 1943	4	6 B	
54	F	30	Mar. 1942	May 1943	21	6 B	
60	F	25	May 1941	Feb., 1943	9	6 B	
65	M.	30	May 1943	Feb. 1943	9	6 B	
69	F	32	Aug. 1941	Feb., 1943	7	6 B	

Average period before relapse discovered approximately 20 months.

Total No. cases (first stage) under observation for over 18 months = 62.

A.B.—6 B 6 T receives 6 injections Bayer 205 and 6 injections trypanamide

20 months. It has always been a matter of routine to follow up cases for a period of at least 24 months. Old cases are called out and examined at each sleeping sickness inspection, and if necessary admitted to hospital for further investigation. Thus these cases which have relapsed have been examined at least 6-monthly after their initial course of treatment, if not 3-monthly.

The relapse rate in first stage cases is very high. It is probable that the course of treatment provided is not sufficient for about 50 per cent. of the first stage cases.

The reason for this appears obvious. The dividing line between the first and second stage case is a positive Pandy's test. If the infection is advanced in the first stage, but has not yet reached the stage when the central nervous system is involved, the Pandy test will be negative, but in such an advanced infection a full course of treatment as for second stage cases is required. The onset and progress of the disease is insidious. A positive Pandy is often obtained before there are any clinical signs of nervous involvement. Thus it would appear that all cases of sleeping sickness should have the same full course of treatment no matter how early the stage of the disease.

In a few selected cases the relapse from first to second stage has occurred after one or two injections of Bayer 205 the drug appearing to activate the trypanosome and for the time aggravate the symptoms. No such cases have occurred at Meridi but these have been noted in Source Yubu.

The long period before the relapse is discovered is probably due to the treatment which effectively controls but does not quite eliminate the infection and obtain a complete cure. The trypanosome then slowly gains the upper hand and ultimately causes a relapse. The possibility of such cases becoming drug-fast is obvious and is another reason for advocating a full course, no matter what the stage of the disease.

Ten deaths are reported (Table VI). One of these (Case 13) is an ordinary African misadventure. Six were in the second stage and of these four had other intercurrent infections. Two second stage cases were resistant to treatment. One first stage case (Case 43) was an infant of 1 year who progressively became worse and died. This is a high mortality rate for sleeping sickness in the Sudan, but having due regard to the fact that this was a fresh outbreak, in many cases the disease was probably in a more advanced stage before it was diagnosed. The relapse rate among the cases found in the first year was over 40 per cent.

The results of treatment are summarized as follows —

(a) A full course of treatment for all cases is desirable if a high relapse rate among first stage cases is to be avoided. The Bayer 205 used was pre war stock but it is not considered likely that the drug would deteriorate so as to be ineffective.

(b) Only one case of blindness and three cases of stomatitis were recorded from the seventy eight cases. These cases were all on a course of trypanamide.

(c) Intercurrent infections and endemic diseases so frequent among African populations, were common among the deaths recorded.

TABLE VI  
DETAILS OF DEATHS

Case No.	Sex	Age	Stage of Disease	Notes
	M.	25		Progressively worse died after 9 months.
13	M.	23	1	Killed by elephant 11 months after infection.
18	F	22	2	Reported died 1 case
29	F	40		Developed stomatitis, and pneumonia during course
40	F	35	1	Died 1 case
43	M.C.	1	1	Relapse to second stage and died 4 months after infection.
49	F	30	1	Died 18 months after course bilharzia and anaemia.
54	M.	55		Resistant to trypanocide and Pandy positive 6 months after course Died 6 months after course
73	M.	35	2	Died from dysentery
76	F	40		stomatitis during course of trypanocide Developed pneumonia. Also infected with bilharzia and malaria. Died during the course

## VII—SUMMARY

1 A localized epidemic of sleeping sickness is described with details of the measures taken to control it and to prevent its spread.

2 Special reference to the control of *G. palpalis* is made, with notes of various experiments carried out. The use of screens in various types of clearings is commented on.

3 The epidemiology of the disease, with special reference to the disease incidence and the fly incidence in the locality is discussed. A household, or "family" incidence of the disease is recorded.

4 The treatment of the cases is detailed, with reference to the high relapse rate in first stage cases.

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## VINCENT'S INFECTION IN NORTHERN PERU

BY

R. VIGORS EARLE, M.D. (LOND.) M.D. (CANTAB.) D.T.M. (SYDNEY) \*

*Medical Director International Equadorian Petroleum Company Guayaquil, Ecuador*  
*Late Jefe-Médico Hospital de Negritos, Negritos Peru*

Infection with Vincent's organisms is widespread in the arid desert region of northern Peru. Commonest among members of the *peon* class it is nevertheless also found among the higher income *empleado* class, though to a lesser degree.

According to LONGHURST (1943) oral sepsis plays an important part in the development of Vincent's gingivo-stomatitis. Other factors are vitamin lack notably that of the B<sub>1</sub> complex (KING 1940) and debilitating diseases (LINTON 1943). All of these factors are present in northern Peru they will now be considered in detail.

### ORAL SEPSIS.

Of 403 patients hospitalized for various complaints, oral sepsis was present as follows —

Dental caries in	241 patients	(= 59.80 per cent.)
Pyorrhoea	207	(= 51.37 per cent.)
Gingivitis	142	(= 35.24 per cent.)

\* I wish to thank Dr. LIONEL W. WIDNEY Chief Geologist, International Petroleum Co. Ltd., for his kindness in supplying me with the meteorological readings for the Negritos Area.



## VINCENT'S INFECTION

The gingivitis described above appeared, clinically to be due to Vincent's infection but facilities were not available to test all of the cases in the laboratory. However from sixty three of the patients who had clinical signs of Vincent's infection, swabs were taken for laboratory examination fifty four (thirty male, twenty four female), or 87.3 per cent. gave positive results.

These Vincent positive cases were studied in detail with respect to the contributory conditions referred to above, namely avitaminosis and concurrent debilitating diseases.

## AVITAMINOSIS

The average age of these fifty four patients was 35.76 years they were of the *mestizo* race, a mixture of Spanish and native Indian, with Indian blood and racial characteristics predominating.

(i) *Developmental irregularity*, held by NICHOLLS (1938) to be in part due to deficiencies in vitamins A and the B complex was present in four (= 7.4 per cent.) of the cases. Of the remainder forty were of average build and one was overweight. However it has been shown (EARLE, 1944) that the average weight, with reference to height and age is lower in the Peruvian than in North Americans, indicating widespread under-development due to faulty diet.

(ii) *Avitaminosis A*—Bitot's spots occurred in two (= 3.7 per cent.) cases nyctalopia was found in seven (= 12.9 per cent.) cases phrynodermis was seen in seven (= 12.9 per cent.) cases. Fine scaling was seen in seventeen (= 31.4 per cent.) cases, being distributed as follows both extremities, six upper extremities, four lower extremities, seven.

Chronic cold cough or bronchitis was present in twelve cases the tonsils were enlarged and septic in twenty four instances.

(iii) *Thiamine deficiency*—Various neuritic manifestations were seen, including cramps, tenderness of the calf muscles, burning of the soles of the feet, numbness of the legs twenty patients complained of such disabilities. Anorexia was present in ten cases and chronic constipation occurred in ten.

(iv) *Ariboflavinosis*—Angular stomatitis (cheilosis) occurred in five cases. Six seborrhoeic disorders were seen as accumulations in the naso-labial folds, Scrotal dermatitis was present in one man.

(v) *Other probable manifestations of lack of vitamin B*—These were coarse scaling of the skin (15 per cent. of the cases examined) hyperpigmented skin areas (34.47 per cent.) hypopigmented skin areas (20.59 per cent.). The last two manifestations are probably but not certainly due to lack of the B complex (EARLE 1942a).

(vi) *Tongue changes*—The following changes due, according to JECHESS (1942) to vitamin deficiencies, were seen dryness and atrophy said to be due to deficiency of nicotinic acid and other factors of the vitamin B complex,

three cases magenta-coloured tongue (possibly due to ariboflavinosis), two cases rawness soreness, fissuring and redness (pellagroid) seven cases

(vii) *Dental changes*—In addition to the changes noted above, dental irregularity and malalignment thought by NICHOLLS (1938) to be due to lack of vitamins A and D in infancy was seen in 20.35 per cent. of the cases studied. Occlusal erosion, principally of the premolars and molars due in part to eating gritty foods and in part, probably to deficient calcium in early life, was seen in 19.11 per cent. of the cases

### CONCURRENT DEBILITATING CONDITIONS

The concomitant morbid states which may or may not have precipitated the development of the gingivitis, were as follows: Work accidents, eleven septic infections (cellulitis axillary abscess etc.) eight malaria (*Plasmodium vivax*), eight childbirth, seven hernia operations three Vincent's angina, three typhoid two, influenza, two cystitis two amoebic dysentery two pulmonary tuberculosis one carcinoma of uterus, one pleurisy one multiple arthritis one syphilis one threatened abortion one.

As has LONGHURST (1943) I have several times seen Vincent's gingivitis develop in the mouths of those receiving bismuth therapy for syphilis

### ABSENCE OF TROPICAL ULCER.

It is now interesting to speculate upon the reason for the complete absence of tropical ulcer in the Talara Negritos area of northern Peru.

I have examined thousands of patients' legs for evidence of tropical ulcer scars without success. Smears from several score leg ulcers—which, however lacked the appearance, shape small (EARLE, 1942b) of tropical ulcer—were negative for Vincent's organisms.

Most of the factors commonly held to make for the development of tropical ulcer are present. DIAZ (1942) has shown the diet in this area to be deficient in proteins, fats and vitamins. There is also a probable lack of calcium as well, though the role of this substance in the production of tropical ulcer is doubtful.

Debilitating diseases said to predispose to tropical ulcer are malaria (APOSTOLIDES 1922), syphilis (MENDELSON 1921) scurvy (APOSTOLIDES 1922) alcoholism and dysentery (SOPRANO 1914) and ankylostomiasis (HUGHES, 1931). In this area amoebiasis is common. Present also is dysentery resembling the bacillary type the identification of which has not yet been undertaken. Syphilis is present but not very widespread malaria is occasional and imported alcoholism is uncommon ankylostomiasis is absent.

Trauma—crushing and bruising of the lower extremities—occurs to the same extent as among the Trinidad oil workers (EARLE, 1942b). Insect bites are present, though to a lesser extent than in Trinidad. Mosquitoes are rare indeed, arriving in small numbers in fruit and other merchandise transported

across the desert by motor lorry. *Anopheles* are found in a small quebrada about 5 miles from the main population centre occasionally extending their range after the brief and often scanty rains (FRAZER 1944 SHIELDS, 1944). In any case, the only persons affected are a few goatherds and peones and even in these I have never seen tropical ulcer.

*Culicoides* and *Phlebotomus* are non-existent. Bites from fleas, bugs, lice and ticks are, however common. Scabies is widespread but even in the scratched areas in the disease, undoubtedly contaminated by fingers which have been in infected mouths, tropical ulcer fails to develop.

CLIMENTS (1936) claims that abrasions, cuts, etc. become infected by saliva, applied accidentally (promiscuous spitting) deliberately (with leaves as a haemostatic) or on the feet or proboscides of flies. Promiscuous spitting is universal in this country (probably accounting in part, for the large amount of tuberculosis) flies abound everywhere—yet tropical ulcer is absent.

### POSSIBLE ROLE OF CLIMATE.

There remains one possible factor to account for the absence of tropical ulcer in this region—climate.

Although only four degrees south of the Equator the climate of this region is temperate, the coastal strip being cooled by the antarctic waters of Humboldt's current. Rain is rare.

It is of interest to compare meteorological readings taken in Trinidad, where tropical ulcer abounds, with those made here, where it is absent. A comparison of the two is made in the Table. The Trinidad readings were made in Port-of Spain and are taken from the report of the Director of Medical Services of that island (1938, 1940). The readings made here were for the year 1940 a typical year as far as climate is concerned.

It will be seen from the Table that every reading made in the Negritos area is lower than the corresponding one made in Trinidad.

Temperatures—maximum, minimum, range and mean—are all substantially lower in Negritos than in Trinidad. The rainfall, as can be seen, is almost non-existent in this part of Peru it is abundant in Trinidad. Relative humidity in Negritos is considerably less than that of Trinidad.

It seems very likely that the absence of tropical ulcer in Negritos is due to climatic factors alone and not to dietetic, traumatic or other morbid conditions.

### SUMMARY

1. An account of the occurrence of Vincent's gingivitis in the Negritos area of northern Peru is given.

2. The relationship between the complete absence of tropical ulcer in Negritos and low temperature, rainfall and relative humidity is indicated.

TABLE.  
METEOROLOGICAL READINGS.

Trinidad							Negritos.						
Month.	Temperature				Rainfall		Month.	Maxi- mum.	Mini- mum.	Range	Mean.	Rainfall.	
	Maxi- mum.	Mini- mum.	Range	Mean	Amount in inches	Relative humidity (mean %)						Amount in inches.	Relative humidity (mean %).
January	92.2	68.7	23.5	80.1	3.66	81.0	January	90	72	18	81	0.012	73
February	93.5	67.3	26.0	78.8	1.94	78.3	February	92	75	17	83	None	75
March	93.5	67.7	26.0	80.6	2.33	77.0	March	93	75	18	81	"	74
April	93.1	68.6	24.5	80.3	4.52	79.2	April	93	74	19	82	0.727	74
May	92.7	70.0	22.0	81.2	3.40	77.5	May	91	71	20	81	None	73
June	92.7	71.3	21.4	81.0	5.10	79.3	June	88	67	21	77	"	70
July	91.6	68.8	22.8	80.7	8.37	84.0	July	81	66	18	75	"	69
August	91.9	70.2	21.7	81.0	9.45	83.5	August	82	63	17	73	"	67
September	92.3	70.8	21.5	81.6	8.51	84.8	September	82	66	16	74	"	68
October	93.5	72.4	21.1	82.0	5.43	83.3	October	81	68	13	74	"	70
November	93.4	72.3	21.1	82.8	11.20	87.3	November	80	67	19	76	"	70
December	91.0	70.5	20.5	79.8	0.90	81.3	December	88	70	18	79	"	74

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## SPINAL ANAESTHESIA IN THE TREATMENT OF TROPICAL ULCERS

BY

EDWARD W. PRICE, M.A. M.B., B.CH. (CAMB.) D.T.M. (ANTWERP)  
*Baptist Mission Hospital Boma Belgian Congo*

Chronic ulcers usually of the leg are one of the bane of a tropical practitioner's life. They incapacitate the patient—usually a native—considerably without his feeling really ill—he gets tired of the long treatment, the nursing staff find it trying, the other patients dislike the smell, and the doctor often seems particularly ineffective.

The difficulties of treatment are evident from the multitude of suggested methods. These vary from exposure to air without dressing, insulin dressings to intraspinal injection of alcohol (JAMES 1939) and putting a bag of common salt in a cauterized ulcer bed and/or potassium permanganate cautery (TODD 1939).

Perusal of the literature and a consideration of one's own experience suggests that the condition must vary considerably from place to place as to its severity and resistance to treatment.

In this district of Central Africa—near Lisala in the Belgian Congo—the following routine has proved sufficient for most cases—

1. Removal of native medicine—often a difficult process.
2. Simple dressings with eusol.
3. Injections with neosalvarsan, if necessary.

4 If these fail, immobilization of the leg in bed with a splint or simply by tying the foot to the end of bed often produces satisfactory results.

5 Change to another antiseptic.

6. Infrequent dressings—only re-dressing when either the patient himself insists, feeling he isn't being properly looked after or the smell becomes quite unbearable.

Only rarely does one have to resort to curetting

There are however occasional ulcers which resist all these forms of treatment, and an experience with two such during the last few weeks prompts this short paper

### CASES.

Case 1—Mondanga, native boy aged about 14. Ulcerations of both feet spreading on to dorsum and plantar aspect of foot from infected toes (jiggers). Admitted to hospital 20.10.43. Eusol dressings, neosalvarsan, immobilization all without effect. The ulceration seemed particularly painful, especially at night, sometimes needing morphia for its alleviation.

The ulcer eventually became stationary showing neither progress nor regression. There was no evidence of deep infection or osteitis sufficient to account for the persistence of the ulcer.

1.12.43 Therapeutic spinal anaesthesia. 75 mg procaine in 1 c.c. sterile water between L3 and L4 the patient then being turned on his back and the knees drawn up on to the abdomen for 1 minute. No other treatment.

6.12.43 Ulcers completely healed. Patient went home.

Case 2.—Ekonde, adult leper aged about 25 with foul smelling ulcers on sole of left foot. No sinuses. Prolonged local treatment at leper village without success. Hospitalized 9.12.43. Neosalvarsan and immobilization without effect.

20.12.43. Therapeutic spinal anaesthesia. 150 mg procaine in 1.50 c.c. sterile water between L3 and L4 the knees then drawn up on to the abdomen for 1 minute, the patient lying on his back.

26.12.43 All ulcers healed. Patient went back to leper village.

### DISCUSSION

The above histories suggest that the rapid healing was the direct result of the spinal anaesthesia.

JAMES (1939) suggested that these ulcers tend to persist indefinitely and that obliterative endarteritis places the lesions outside the realm of ordinary medical treatment.

The above experience suggests that there is a preliminary prolonged arteriolar spasm at the base and vicinity of the ulcer which is the cause of the chronicity.

The inefficacy of injections of neosalvarsan in cases where there is a definite syphilitic or yaws or other spirochaetal basis (and where in consequence one would expect a satisfactory response) may then be related not necessarily to an accompanying endarteritis but to a superimposed arteriolar spasm which prevents the drug from reaching the spirochaetes.

This possibility suggests that in such cases some method of relaxing the arterioles concurrently with injections of neosalvarsan would increase their efficacy.

MONTGOMERY (1942) compares the various methods of increasing blood-flow in the extremities and concludes that the maximal effect is obtained by one of six methods: heat to body without fever; artificial fever; anaesthesia (general, spinal or paravertebral); periaarterial stripping; and preganglionic sympathectomy, though the duration varies in ascending order.

Such considerations were at the basis of the attempt to heal the ulcers in the above cases by simple spinal anaesthesia.

The results were dramatic and impressive and though it is probable that all chronic ulcers will not respond in this way there is sufficient justification for drawing attention to its use either as the main method of treatment, as in the above cases, or as an adjuvant to arsenical injections.

The apparent objection—that spinal anaesthesia with procaine is of short duration—did not seem to affect the rapid healing in the cases cited, and one draws the conclusion that the effect was one rather of breaking a reflex than of maintaining a vasodilatation.

Such an experience is similar to that of OCHSNER (1939) who finds that the clinical manifestations of thrombophlebitis are immediately relieved—and remain so—with paravertebral block with novocaine.

It is remarkable that the reflex does not seem to re-establish itself again in view of the fact that the provocative element, presumably the thrombophlebitic vessel, remains as it was before the anaesthesia.

It would seem, then, that either anaesthesia (spinal or general, according to MONTGOMERY) or paravertebral sympathetic block with an anaesthetic is sufficient to cause vasodilatation of sufficient length to remove permanently the symptoms of a phlebitis or permit the rapid healing of a chronic ulcer. The length of the anaesthesia in hours seems to be of secondary importance.

#### SUMMARY

- 1 Two cases of chronic ulcers of the leg in natives in the Belgian Congo are described.
2. After other methods had failed, rapid healing took place after a spinal anaesthetic (procaine) without further local treatment.
3. A comparison is drawn with other conditions, e.g. thrombophlebitis where similar rapid therapeutic effects are obtained.



4. It is suggested that spinal anaesthesia in conjunction with arsenical injections would hasten the healing by permitting a greater blood flow of arsenic-bearing blood to the tissues in cases where there is a spirochaetal basis for the chronicity.

5. Non-spirochaetal ulcers seem to improve merely by the increased blood-flow following spinal anaesthesia.

6. It is remarked that the reflex once broken does not seem to re-establish itself even when the original provocative focus remains.

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## A STAINING METHOD FOR MALARIA PARASITES IN THICK BLOOD FILMS

BY

J C B FENTON R.A.M.C.\*

AND

JAMES INNES F.R.C.P.E. MAJOR, R.A.M.C.

*From a Malaria Research Team, C.M.F.*

Much work has been done to improve and simplify the staining methods employed on thick blood films used in the diagnosis of malaria infections. Within the last few years a considerable advance was made by the introduction by FIELD† of a rapid staining technique which has been widely adopted in many laboratories. The purpose of this paper is to describe a modification of Field's staining method, which has been found to ensure that optimal staining is more regularly achieved.

Whereas the pH of Field's stain is 6.6 in this stain a pH of 6.8 has been found necessary since the film becomes dehaemoglobinized during staining.

\* We wish to record our gratitude to Brigadier E. R. BOLAND O.B.E. Consulting Physician, A.F.H.Q., for his encouragement and support in providing us with the opportunity to carry out this work, and for granting us permission to publish this account of one aspect of our investigations.

† FIELD J. W. (1941) Further note on a method of staining malaria parasites in thick blood films. *Trans. R. Soc. trop. Med. Hyg.* 35 1 33-42

The haemoglobin that tends to absorb the eosin is thus removed and a less acid stain is required to give the optimum results.

The following are the directions for the preparation and use of the stain

#### PREPARATION

There are three staining solutions—A B and C A being the methylene-blue azure solution, B the eosin stain, and C a corrective Leishman solution.

*Stain A* Is made from a mixture of two solutions which should be prepared separately

Solution (i)	Methylene blue	1 gramme.
	Disodium hydrogen phosphate ( $12 \text{ H}_2\text{O}$ )	5 grammes.
	Distilled water	250 c.c.

Place this solution in a 500 c.c. conical flask and immerse in a bath of boiling water for 2 hours 15 minutes. The neck of the flask should not be plugged, but allowed to have free access to the air as the formation of azure depends on the oxidation of alkaline methylene blue by the atmospheric oxygen. If the azure is prepared under these conditions, the right proportion of azure in relation to the methylene blue will be formed. Azure made by this poly-chromizing method has been found more satisfactory for staining than pure Azure 1 added to methylene blue in the preparation of this stain. The poly-chromizing should not be carried on beyond the stated time or too much azure will be formed, and too much is almost as bad as too little. If the azure is in excess, then fibrin threads will stain in the background of the films, the parasite cytoplasm will not stain a good blue colour and the parasite chromatin may appear black.

A heavy deposit may separate out when the solution is heated and the flask should be set aside, say overnight, to allow this to re-dissolve. When cold, the solution should be made up to its original volume and should appear a purple blue colour when viewed in artificial light. Too much azure production results in a more reddish purple colour

Solution (ii)	Methylene blue	1 gramme.
	Potassium dihydrogen phosphate	2 grammes.
	Distilled water	500 c.c.

To prepare Stain A add two parts of Solution (i) to one part of Solution (ii). This method of preparation ensures that there is always some unchanged methylene blue present in Stain A rather than a mixture of poly-chromed by products.

<i>Stain B</i>	Yellow water soluble eosin	1 gramme.
	Disodium hydrogen phosphate ( $12 \text{ H}_2\text{O}$ )	3.3 grammes.
	Potassium dihydrogen phosphate	1.3 grammes.
	Distilled water	500 c.c.

The pH of the above Stains A and B should be approximately 6.8.

*Stain C* A stock buffer solution is required to prepare this stain and consists of —

Disodium hydrogen phosphate ( $12 \text{ H}_2\text{O}$ )	12.5 grammes
Potassium dihydrogen phosphate	5 grammes.
Distilled water	250 c.c.

Ether, 0.5 to 1.0 c.c. may be added to preserve this solution from bacterial contamination

The stock buffer solution should have a pH value of approximately 6.8. This should be checked with an indicator if there is any reason to suspect that the sodium phosphate has lost some of its water of crystallization.

To prepare the corrective Leishman stain, make a 1 in 50 dilution in distilled water of ordinary 0.15 per cent. Leishman stain, and add the stock buffer solution at the rate of three parts in 100 of the total mixture.

#### METHOD OF USE.

The staining times for a thick blood film are recommended as follows although these can be varied a great deal without producing an adverse result

- 1 Dip in Stain A for 1 to 2 seconds.
- 2 Wash in distilled water for 1 second.
- 3 Dip in stain B for 2 to 3 seconds
- 4 Wash in distilled water for 1 second.
- 5 Place in stain C for 10 to 15 minutes in a small staining dish.

The slide should be taken out of the Leishman stain and stood upright to dry without further washing in water

#### NOTES ON THICK FILMS STAINED AS ABOVE.

1 No definite specifications as to size or thickness of the blood film need be laid down for staining by this method. The films can be much thicker than those recommended for use with Field's technique as dehaemoglobinization occurs as in the dilute Romanowsky staining method.

2. The naked eye appearance of a successfully stained film is that it should be completely transparent and without any residual haemoglobin. The colour should be a very faint purple blue

3 After staining, the film may be dried in an incubator if desired but like other Romanowsky stains, if dried by direct heat it will turn blue.

4 No harm is done by leaving films for much longer than the specified 10 to 15 minutes in the dilute Leishman stain. In very thick films such longer time will produce better results by ensuring complete dehaemoglobinization.

5 Films that turn blue on drying have either

(a) not been completely freed of haemoglobin and should have been left longer in the Leishman stain or

(b) have been differentiated in a Leishman stain more alkaline than pH 6.8

6 Films that have an excessively red appearance have been differentiated in a Leishman stain more acid than pH 6.8.

*The Microscopical Appearances of a Successfully Stained Thick Film.*

The stained leucocytes, platelets and malaria parasites are contrasted against a clear white background, thus resembling in general a film well stained by dilute Giemsa. Since the methylene blue azure solution in Stain A has a mild fixative action, there is little or no distortion of the leucocytes. The granules of the cytoplasm of the polymorphonuclear cells are often powerfully stained. In *Plasmodium vivax* infections the Schuffner's dots usually stain intensely in the thinner parts of the film. This gives rise to the "ghost cell" effect that is seen also with Giemsa stain and when present forms a diagnostic indication of the benign tertian parasite that may be most valuable evidence in a case where young ring forms are seen only in the film.

The parasite cytoplasm is stained a blue or grey blue colour and fine details in its structure can often be seen. The parasite chromatin stains a deep red.

SUMMARY

1 A staining method for thick blood films is described employing methylene-blue-azure coum and a dilute Leishman stain.

2 It is claimed that this modification of Field's method ensures optimal staining results for the demonstration of malaria parasites.

TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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VOL. XXXIX. No 2. OCTOBER, 1945

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THE THIRTY-EIGHTH ANNUAL GENERAL MEETING

of the Society held at

Manson House, 26, Portland Place, London, W 1,

on

Thursday, 21st June, 1945, at 8 p.m.,

THE PRESIDENT

SIR HAROLD SCOTT K.C.M.G., M.D. F.R.C.P. F.R.S.E.  
in the Chair

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BUSINESS

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REPORT OF THE COUNCIL FOR THE YEAR ENDED 31st MARCH 1945

The Hon Secretary, Dr WENYON presented the Annual Report, which  
had been circulated to the Fellows present at the meeting

The Report was unanimously adopted.

REPORT OF THE HON TREASURER FOR THE YEAR ENDED 31st MARCH 1945

The Hon Treasurer, Dr MARRIOTT presented his Report, with the  
Accounts and Balance Sheet prepared by the Auditors and accepted by the  
Audit Committee

The Treasurer's Report was unanimously approved and adopted

MANSON HOUSE.

The Hon. Treasurer (Dr MARRIOTT) made a statement regarding the  
Manson House Fund.

He recalled that it had been started in 1923 for the purpose of buying  
a house to serve as the Society's headquarters and at the same time constitute  
a memorial to Sir PATRICK MANSON

Donations had been received from members of the Royal Family from Colonial Governments from commercial firms and others interested in the tropics, and from over a thousand Fellows of the Society.

After much searching a suitable house was found and in 1931 26 Portland Place was purchased and named MANSON HOUSE.

The total expenditure, including alterations building the Lecture Hall and furnishing was nearly £30 000. To meet this in 1932 a loan on mortgage of £15,900 had to be arranged. Part of the premises were let and the rents received helped to reduce the debt steadily year by year.

In 1940 our tenant's lease expired and was not renewed as the premises had been badly damaged by bombs. However in 1943 we were able to get the damage repaired and secure good tenants. Once again it was possible to make substantial reductions in the debt, and a legacy of £5 000 from Mrs. M. H. COLDWELL speeded the process.

By 31st March, 1945 only £1 02s remained on loan. The Treasurer said that the Council at its meeting in May 1945 had decided to pay off the whole of this remaining debt, utilizing £600 from the balance on 31st March and £425 from subscriptions and rents received since that date. He was therefore now able to announce that Manson House was entirely free from debt.

The Treasurer said he could not let this auspicious occasion pass without referring to the unwavering support and financial help given by Dr. CARSHILLAL LOW one of the chief originators of the scheme and to Dr. WENTON who, as Hon. Secretary had watched and directed the whole idea of Manson House. He also spoke of Miss WENTON's active participation throughout and her refusal to be deterred by all kind of difficulties, not overlooking the difficulties caused by enemy action.

#### ELECTION OF THE AUDIT COMMITTEE

Dr W. E. COOKE, Dr J. C. BROOM and Dr C. RUSSELL AMES were re-elected as members of the Audit Committee.

#### ELECTION OF PRESIDENT TWO VICE PRESIDENTS AND TWENTY COUNCILLORS FOR 1945-47

The PRESIDENT announced the result of the Ballot as follows —

##### *President*

\*C. M. WENTON C.M.G. C.B.E., M.B. B.S. B.Sc. F.R.S.

##### *Vice Presidents*

\*D. B. BLACKLOCK, C.M.G. M.D. D.Ph. D.T.M., Professor

\*JOHN A. SINTON V.C. O.B.E., M.D. D.F.C. B.Ch. Brigadier I.M.S. (ret.)

*Councillors*

- A R. D. ADAMS M.D., CH.B. M.R.C.P., D.T.M.  
 \*A G. BIGGAM C.B. O.B.E., K.H.P. M.D. F.R.C.P. Maj.-Gen. late R.A.M.C.  
 \*P. A. BUXTON M.R.C.S. L.R.C.P. D.T.M. & H., F.R.S. Professor  
 C. C. CHESTERMAN O.B.E., M.D. B.S. M.R.C.P. D.T.M. & H.  
 \*T. H. DAVEY O.B.E., M.D. D.T.M. Professor  
 W. R. M. DREW O.B.E., M.B. B.Sc. F.R.C.P., D.T.M. & H. Lt.-Col. R.A.M.C.  
 N. HAMILTON FAIRLEY C.B.E., M.D. D.Sc. F.R.C.P. F.R.S. Brigadier R.A.M.C.  
 R. M. GORDON O.B.E., M.D., M.R.C.P. D.P.H. D.T.M. Professor  
 \*H. M. HANSCHALL D.Sc. M.R.C.S. D.T.M. & H.  
 R. BRUNEL HAWES M.B. B.S. F.R.C.P.  
 \*E. H. VERE HODGE C.I.E., M.D. F.R.C.P. Lt. Col. I.M.S. (ret.)  
 W. H. KAUNTZE, C.M.G. M.B.E. M.D. CH.B. F.R.C.P.  
 GEORGE MACDONALD M.D. CH.B. D.P.H. D.T.M.  
 Sir PHILIP MANSON BAHR, C.M.G. D.S.O., M.D. F.R.C.P. D.T.M. & H.  
 OSWALD MARRIOTT M.D. B.S. M.R.C.P.  
 F. MURGATROYD M.D. F.R.C.P. D.T.M. Lt. Col. R.A.M.C.  
 \*Sir JOHN TAYLOR, C.I.E., D.S.O. M.D. D.P.H., Major-General I.M.S. (ret.)  
 F. NORMAN WHITE, C.I.E., M.D., I.M.S. (ret.)  
 Sir HAROLD WHITTINGHAM, K.C.B. K.B.E. K.H.P. F.R.C.P. Air Marshal R.A.F.  
 CHARLES WILCOCKS M.D. CH.B., M.R.C.P. D.T.M. & H.

## \*New Nomination.

The President (Sir Harold Scott) Fellows of the Society 2 years ago I reached the acme of my career when you did me the honour of electing me President of our Society. I promised then that I would do my utmost to maintain its prestige, and that promise that resolve I have endeavoured to carry out. Today my term as President comes to an end but before handing over the reins of office to my successor it is my duty to give you very briefly an account of my stewardship in a short valedictory address. You have read and heard the Annual Report which tells you of our doings during the past year and of our present standing and you have expressed your approval of it. You have heard also the Report of the Hon. Treasurer which shows the very satisfactory state of our finances, and our best thanks are due to Dr MARRIOTT for the zeal and acumen with which he has performed the duties of his position and brought things to such a happy pass. With regard to the debt on Manson House that once stood at what the journalists are pleased to call an astronomical figure a little over a month ago it had become so much reduced that I thought it would be my privilege to tell you that in all probability my successor would have the felicity of announcing our total liberation from debt. As you have heard from Dr MARRIOTT by a happy concatenation of circumstances—may



I call it that? I have been able to jump his claim and announce today myself, our release from this burden.

During the past few years a considerable number of our Fellows have been the recipients of honours and awards. Our heartiest congratulations go out to them one and all. I will not enumerate them because although I have got together a goodly list, I may have missed some, and they would naturally feel hurt at being left out. During the year there have been thirteen resignations, and we have lost fifteen others by death some in the ordinary course of nature, others, alas, as the result of enemy action, all too early in their career. I will not enumerate these either they are mentioned in the Annual Report but I would like to say a few words on one or two of them.

FREDERICK PERCIVAL MACKIE held all the highest British medical qualifications. He was a Fellow of the Royal College of Physicians and a Fellow of the Royal College of Surgeons. His name had been before the medical world prominently since the beginning of the century. In 1903 he was with the Younghusband mission to Tibet. He was engaged in plague work in India in 1905 with the Uganda Sleeping Sickness Commission in 1908, and with the kala Azar Investigation in Assam in 1913. Other positions which Colonel MACKIE held included those of Professor of Pathology at Calcutta, Director of the Pasteur Institute, Assam, and of the Haffkine Institute, Bombay. He was Health Commissioner for the Government of India in 1928, Director of the Pasteur Institute and Research Laboratory at Shillong and, at the time of his death, Chief Medical Officer British Overseas Airways Corporation. He had also been King's Honorary Surgeon. We should have to go far to find a record to equal his.

BURGESS BARNETT had a wide reputation as a herpetologist. His name came still more prominently before the medical public because of his work on the application of snake venoms to general medicine, in relief of pain in cancer, tabes, leprosy and in haemorrhagic conditions. He died on service in Burma.

JOHN CHARLES GRANT LEDINGHAM was not a very active Fellow of our Society during recent years, but formerly he was closely associated with our work. He had, as you all know, an international reputation as a bacteriologist. He succeeded Sir CHARLES MARTIN as Director of the Lister Institute and was Chairman of the Tropical Diseases sub-committee of the Royal Society. The loss to the Colonial Medical Service and to our own Society by the death at enemy hands of EDMUND CYRIL SMITH I spoke of on a former occasion. Of the others I will not say anything special except that, if I may, I will quote the words written over 2,000 years ago and there is authority for saying that even the devil can quote Scripture to his purpose. Some there be that have no memorial but their glory shall not be blotted out, nor their righteousness forgotten, their name liveth for evermore and people will tell of their wisdom."

During the year as you heard from the Annual Report, there have been seventy new Fellows elected to the Society. The number should be greater

and I cannot understand why it is not. It cannot be that the provision we give is poor. That the mental pabulum is adequate and acceptable is, I think, evidenced by the large attendance at our meetings. Of the Colonial Medical Service a fair proportion join as Fellows but of other Services the Royal Naval Medical Service the Royal Army Medical Corps the Indian Medical Service and the Royal Air Force Medical Service it must be confessed lamentably few. Our meetings have been more frequent this year than previously and on the whole better attended. On more than one occasion we have had a record attendance and late comers have been hard put to it to find a seat. Many papers of great interest have been read, many excellent discussions have been held and many other contributions would have been delivered but could not be for lack of time. It would be invidious to select any of these the list is given in the Annual Report which you have seen but I must just refer to one the historic meeting which we held here on the 14th of December last in celebration of the centenary anniversary of the birth of Sir PATRICK MANSON when we had the privilege of hearing an address absorbingly interesting delivered by MANSON'S son in law Sir PHILIP MANSON BARR, which he rightly entitled the Manson Saga. If I may digress for a moment I may say that I never met MANSON. When I entered the Colonial Service I was told to call and see him and be examined. I went in fear and trepidation, for his name was one to conjure with and we novices looked upon him as a god. I called but the god, like Baal of old, refused to answer. Quoting again from the same source as before I might say perhaps he was talking or he was pursuing peradventure he was sleeping anyhow I had to go to his able coadjutor Dr C W DANIELS. Nevertheless throughout my tropical career the name the work, the character the discoveries of MANSON were a constant stimulus to me in my small way. I always felt that nothing shoddy must be allowed to pass as good, surmise must never do duty for fact, that every reference must be verified. When I came back from the tropics I was ill and his son-in-law took me at once into hospital. MANSON too was a sick man. Although messages passed between us MANSON died while I was still in hospital and thus I never met him. Later whenever I have entered this building I have felt that it is under Manson's aegis and I therefore enter it somewhat as though I was treading on holy ground because it is dedicated to him and bears his name and I think I may add that I never leave it without the thought, the silent question, Have I said or done anything which I should have regretted saying or doing had MANSON himself been present?

Before I come to the induction of our new PRESIDENT I would like to express my very sincere thanks firstly to the members of the Council, individually and collectively for their loyal co-operation help and advice to the members of the Executive Committee for more than I can express to the Honorary Treasurer for keeping our heads above water and piloting us through a difficult time, and now as regards the debt on Manson House bringing us to such

a happy issue out of all our afflictions—to the Secretaries and every one of the secretarial staff for their affability, cheerfulness and encouraging help, all of which do so much to make a routine which must at times be irksome, pass smoothly. Lastly but far from least, to every one of those Fellows who have given me of their friendship, a friendship which I assure you I value very highly and loss of which I feel all the more poignantly now the time has come to say farewell.

I have now reached the point for which you have waited so patiently, the induction of our new PRESIDENT Dr C M WENYON. This is a belated election. Dr WENYON should have been PRESIDENT a dozen years ago but time after time he has refused it for altruistic reasons, saying that he felt he could be of more use to the Society by staying as its Honorary Secretary and piloting others along the way in which they should go. At long last he has been prevailed upon to take his rightful place. Dr WENYON needs no introduction from me, you know him as well as I do. Is it not strange that the greatest men of science go down to posterity with their names attached to such small things—SCARPA with his triangle, HUNTER with his canal, SYLVIVS with his fissure, FALLOPIUS with a couple of tubes, VESALIUS and WENSLÖW by a foramen each? But our Fellows are noted for far more tangible things than these small though they may be. Not merely by a geometrical figure as SCARPA, by holes like VESALIUS and WENSLÖW or by mere conduits like HUNTER and FALLOPIUS. We have BRUCE known by a whole genus of bacteria and by a trypanosome, CHRISTOPHERS and JAMES by their mosquitoes. Imitation is the sincerest form of flattery and there is even an *Anopheles pseudo-jamesi*. What more could you want? We have LEIPER with his worm, two worms, it may be more. Now WENYON with something quite definite but whose nature is still, I think, *sub judice* namely *Eperythrozoon wenyonii*. For more than a decade there has been a discussion as to whether it is not *Bartonella wenyonii* or even *Haemobartonella wenyonii* or whether it is a protozoon at all. Here we have the name of one of the greatest protozoologists—to spare his blushes I do not say the greatest—whose name goes down to posterity attached to something whose genus is unknown and whose place in the natural order is undetermined. Still there it is, the name has been given, and as long as the laws of nomenclature hold fast that name will stay. *Eperythrozoon wenyonii* may change its generic name its surname, as often as a fashionable movie star but the specific, the Christian name of *wenyonii* has been bestowed in perpetuity.

Ladies and gentlemen I have finished. It remains for me to induct Dr WENYON as our PRESIDENT and to invest him with the badge and chain of office. Rex mortuus est, vivat Rex! May his reign be as happy and fortunate and prosperous as you have made mine.

Sir HAROLD SCOTT then invested Dr WENYON with the PRESIDENT's badge and chain of office.

The President (Dr C M Wenyon) Ladies and gentlemen I must thank you very sincerely for the cordial reception you have given me. And now that I have been transferred by Sir HAROLD SCOTT to the Presidential Chair I want to thank you Fellows of the Society everywhere for conferring upon me the great honour of electing me your President for the coming two years. After Sir HAROLD SCOTT's most eloquent utterance, and in view of his profound knowledge of tropical medicine I have a feeling of diffidence in taking over from him the duties of President. I think Sir HAROLD SCOTT first entered the field of tropical medicine when he became Government Pathologist in Jamaica in the early part of the last war. Before that he had served in the South African war as Captain in the R.A.M.C. and before that he had been at the Cambridge Hospital Aldershot. In Jamaica he threw himself into the work he had in hand with that vigour and enthusiasm which he has displayed in everything he has undertaken. He interested himself particularly in the malarious vomiting sickness and finally solved that difficult problem. He investigated epidemics of typhoid fever and also studied the intestinal protozoa. He might have continued these studies and become a distinguished protozoologist. From Jamaica he was transferred to Hongkong where he still further extended his experience of tropical medicine. He devoted himself particularly to tuberculosis, and published some most valuable reports on this disease in the native population. He then took up the subject of sprue—perforce this may have been, because he was able to study the malady in his own person. He evolved a new theory which, although it may not have been substantiated nevertheless stimulated interest to such an extent that researches on sprue were revived and have gone on regularly since then. Sir HAROLD SCOTT was invalided from Hongkong and was able to continue the study of the disease in this country. Later he took up the study of helminthology and worked in Professor LEIPER's department at the London School of Hygiene and Tropical Medicine. There he carried out investigations and experiments on the tape worm *Hymenolepis nana*. After that he was appointed Pathologist to the Zoological Society. It was then that I first came into close contact with him and realized how indefatigable he was in every work to which he set his hand. I had undertaken the task of examining blood films of all animals that died in the Zoo and Sir HAROLD and I were thrown together very closely in this work. Later on, after this very varied experience, Sir HAROLD SCOTT came into his own when he was appointed Assistant Director at the Bureau of Hygiene and Tropical Diseases under Sir ARTHUR BAGSHAW. By this step he entered the literary field in which he is so distinguished. When Sir ARTHUR retired Sir HAROLD SCOTT became the Director and a great deal of the success of the Bulletins issued by the Bureau is due to Sir HAROLD SCOTT's skilful and enthusiastic editorship. His wide knowledge combined with a literary flair led him to write a number of books. One of these was *Notable Epidemics* another

*Health Problems in the Empire* and yet another a most notable one, *Tuberculosis in Man and Lower Animals*. Finally I think it was in 1937 or 1938, fortunately for us he was invited to deliver the FitzPatrick lectures at the Royal College of Physicians, and chose as his subject the History of Tropical Medicine. These lectures were so much appreciated that he was induced to expand and arrange them in book form and there appeared in due course that immortal work, *SCOTT'S History of Tropical Medicine*. A new edition has already appeared and every one who requires information about the history of tropical medicine and the early workers in this field, turns instinctively to Sir HAROLD SCOTT'S great book of reference. After all the experiences and investigations in which Sir HAROLD SCOTT played such an important part, he was peculiarly fitted to be PRESIDENT of this Royal Society. On that account, therefore I feel some diffidence in taking over the duties from him. I hope, however you will forbear with me during my Presidency and I think I can do no better than promise you, as Sir HAROLD SCOTT has told us he determined when he became PRESIDENT to do my best for the welfare and prestige of the Society while I hold this office.

- It is the first duty of a new PRESIDENT to appoint a Vice President. According to the rules of the Society the Fellows of the Society elect the President two Vice Presidents, and the Members of Council, who are nominated by the retiring Council and it is the prerogative of the new PRESIDENT to nominate one Vice-President himself. Accordingly I have the greatest pleasure in nominating Dr NORMAN WHITE to be my Vice-President during the period of my tenure of office. He has served on the Council for a number of years, has been indefatigable in his attendance, and has contributed to the work of the Society in many ways.

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At the first meeting of the new Council on July 12th, Col. H. E. SHORTT C.I.E. M.D. F.R.S. (ret.), was elected a Member of the Council in place of Dr NORMAN WHITE who had been appointed a Vice-President.

## COMMUNICATIONS

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### LATE RESULTS OF TREATMENT OF SLEEPING SICKNESS IN SIERRA LEONE BY ANTRYPOL TRYPARSAMIDE PEN- TAMIDINE AND PROPAMIDINE SINGLY AND IN VARIOUS COMBINATIONS

BY

R. D. HARDING D.M. M.R.C.P.,  
*Trypanosomiasis Service Sierra Leone*

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#### INTRODUCTION AND METHODS

Records of observations on sleeping sickness patients followed up individually after treatment in adequate numbers over a sufficient period of time, and controlled by lumbar puncture so as to make a sound evaluation of the final cure rate possible, are very scanty in the literature. This paper describes an attempt to estimate the percentage of final cures after the administration of various drugs singly and in combination in various stages of the disease as met with in the Kailahun District of Sierra Leone. The courses of treatment whose effects have been studied comprise —

\* 1. Antrypol, five doses of 1 gramme at 5-day intervals to cases with a normal cerebrospinal fluid cell count.

12. Tryparsamide six to 10 doses of 2 grammes at 5-day intervals. The majority of patients received nine or ten doses.

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\* The metric system is used throughout this paper in stating dosages.

- iii. Antrypol three doses of 1 gramme, followed by trypanamide three to five doses of 2 grammes, all at 5-day intervals.
- iv. Three other combinations of antrypol and trypanamide designed to reduce the toxicity exhibited by Course iii. They have been included in one group for analysis as the cases concerned are too few for separate consideration.
  - (a) Antrypol, three doses of 1 gramme followed by trypanamide three to seven doses of 2 grammes. An interval of 5 or 7 weeks separated the first and second doses, the remainder being given at 5-day intervals.
  - (b) Antrypol, two doses of 1 gramme, followed by trypanamide four to six doses of 2 grammes at 5-day intervals.
  - (c) Antrypol, one dose of 1 gramme followed by trypanamide eight or nine doses of 2 grammes, at 5-day intervals.
- v. Pentamidine eight to twelve doses of 50 to 100 mg daily
- vi. Proparsidine eight doses of 50 or 75 mg daily

The remaining three courses of treatment, whose results are dealt with in Part II constituted a small-scale trial with a combination of pentamidine and trypanamide administered concurrently

- vii. Pentamidine, five doses of 100 mg., and trypanamide five doses of 2 grammes, at 5-day intervals. On each treatment day both drugs were given, the pentamidine first, and trypanamide about 2 hours later.
- viii. Pentamidine five doses of 100 mg. daily and trypanamide five doses of 2 grammes at 5-day intervals. Trypanamide was given on the first treatment day, pentamidine on the 2nd to 6th days inclusive and trypanamide again on the 7th, 12th, 17th and 22nd days.
- ix. Trypanamide alone five doses of 2 grammes, at 5-day intervals to serve as a control for Courses vii and viii.

The doses stated were those given to patients who weighed 100 lb or over. For others the dosage given bore the same ratio to the full dose as did the patient's body weight to 100 lb. In the great majority of cases the drugs were administered by intravenous injection, in a few they were given intramuscularly.

It will be noticed that the total dosage given to different patients treated by Courses ii, iii, iv and v varied considerably. However a study of the results obtained by the higher and lower dosages in each course revealed no significant difference and the cases concerned have therefore been grouped together to facilitate statistical analysis.

In the whole area in which patients were followed up 2,713 had received treatment by one or other of the courses listed. Of these, 228 had died, 1,800 were actually seen and examined, 559 were traced and definite information as to their whereabouts and condition was obtained, and 126 were untraced. A cell count had been carried out on the cerebrospinal fluid before treatment in 1,170 patients and in 962 it was performed in the final survey when a total protein estimation was also made in the great majority of cases. The patients whose fluid was examined both before treatment and at the final survey numbered 579. The interval between the beginning of treatment and the survey varied in individual cases between the extremes of 13 and 28 months, but the average interval for the different treatment groups varied only between 14 and 25 months. The mean interval for all cases was 17 months. Some

three score cases who gave a borderline cell count at the survey were lumbar punctured yet again at anything up to 46 months after treatment.

None of the patients had received any treatment prior to the courses studied except about a score included in Course II (trypanamide only). These few cases had been previously treated by a diamidine and had obviously relapsed with clinical symptoms and a high cell count when seen a few months later. Such cases have also been included in the appropriate diamidine group where the cell counts given after treatment are those which were obtained at the time of relapse and not, as in all other cases at the time of the final survey. To have omitted them would have resulted in weighting the diamidine results too favourably.

#### PROCEDURE.

The survey unit consisted normally of the writer and his wife, an African interpreter and a number of African microscopists. Dr I. APTED of the Colonial Medical Service assisted in part of the survey and continued it for a few days in the writer's absence. Suitable centres in the area were chosen and all previously treated patients who were available were called in to one or other of these centres together with the section and village chiefs concerned to give corroborative evidence as required. Each patient was identified and subjected to a questionnaire which included the following: time and number of injections received to confirm the treatment record; present state of health; presence or absence of headache, body pains, backache, somnolence, itching of the skin, disturbance of vision, ability to perform normal work. The histories of patients reported dead were enquired into in detail in order to ascertain the approximate date of death and its probable cause. After each patient present had been questioned, his neck was palpated, and if cervical glands were palpable one was punctured. If none were palpable or if gland puncture proved negative a thick blood film was prepared, stained with Giemsa, and examined for 10 minutes. At first blood films were prepared from every patient, but when after some hundreds of patients had been examined it was found that trypanosomes were hardly ever to be seen even in severe clinical or CSF relapses, this procedure was limited to about half the cases, but it included all patients whose appearance or history was in the slightest degree suggestive of relapse or reinfection. In all blood films were examined in about two-thirds of the patients seen. Finally lumbar puncture was carried out on as many of the patients as time allowed, but care was taken that they should be a random sample of the cases in each treatment category. A cell count was then made on the cerebrospinal fluid, using a Fuchs-Rosenthal counting chamber and the total protein was estimated by Mestrezet's method. In doing the count, the cells in 1 c.mm. were first enumerated, and if they fell below 4 or above 8 this count was taken as final; otherwise, a further 2 c.mm. were counted, up the same preparation and the average of the three



counts was taken. All C.S.F. examinations were made by the writer or Dr APTED.

In setting out the results preparatory to analysis the patients were classed under treatment course and cell count, if known, before treatment, and then subdivided into the following categories. If alive into Received insufficient treatment, *i.e.*, less than the minimum dosages stated above. Untraced. Traced but not examined. C.S.F. of 0-5 5-10 and 10+ cells on re-examination. Seen but not lumbar punctured. Dim vision. Blind. Trypanosomes seen. If dead into Died during treatment. Died within 3 months after commencing treatment. Died apparently from sleeping sickness at 3 to 12 months. Died after 12 months. Died at any time from unrelated causes.

In calculating the percentage deaths for tabulation the total cases treated have been taken as the denominator. Those who received insufficient treatment are included because in some cases the course was interrupted by toxic reactions and death attributable to the treatment itself. Those who were untraced are included because if they had remained uncured it is unlikely they would have been able to undertake distant journeys and so become lost sight of even had they gone far afield and died, the fact of their death would rapidly have become known to their relatives at home. It is fair to assume, therefore, that such cases had remained alive. Insufficiently treated patients actually only numbered 69 or 2.5 per cent. of the total, and the untraced 126, or 4.6 per cent. In the ideal investigation all patients would be lumbar punctured before treatment, and this procedure would be repeated in all survivors at least 18 months after treatment, but such an ideal can never be realized in practice, and the present investigation probably gives results just about as dependable as one can hope to attain.

#### CRITERIA OF CURE.

The possible criteria to be considered in assessing results are (i) Clinical condition. (ii) presence or absence of trypanosomes. (iii) cerebrospinal fluid.

The greatest debt is due to Dr E. M. LOURIE, who carried out the original investigation and treatment of a large proportion of the cases who have been re-examined, and was responsible for the initial cell count in the majority of cases lumbar punctured before treatment. He has already reported on the C.S.F. findings in his cases up to 4 to 5 months, and on the clinical condition at 12 to 20 months after treatment (LOURIE, 1943), and the present enquiry may in large part be regarded as a continuation of his work to a stage at which a closer estimate of the final cure rate is possible.

Acknowledgment is also due to various medical officers of the Trypanosomiasis Service who carried out treatment in the remaining cases to Dr I. APTED, who assisted at the final survey and to the writer's wife who carried out the questionnaire and compiled the case records. Lieut-Colonel B. G. MARGRAITH kindly prepared and supplied the standard tubes for Mestrezet's test. Dr CHARLES WILCOCKS, Director of the Bureau of Hygiene and Tropical Medicine sent the writer figures and references concerning C.S.F. alterations in yaws. Dr W. J. MARTIN reviewed the statistical data. Messrs. MAY and BAXON supplied stocks of the diamidines. Finally acknowledgment is due to Dr W. P. H. LIGHTBODY C.M.B. Director of Medical Services, Sierra Leone, for permission to publish this paper.

picture particularly cell count and total protein (iv) mortality Their respective value in this survey will now be considered.

(i) *Clinical condition* A clinical history and superficial examination were found to be a poor guide. Some patients with a cell count of above 20 appeared cured clinically and conversely a number with normal counts appeared ill but on more detailed examination proved to be suffering from hookworms, chronic malaria, amoebic dysentery etc. For these reasons clinical condition as a criterion of cure is omitted from consideration in estimating cure rates. It is important to note however that very few patients with cell counts of 20 or less appeared clinically still to be suffering from sleeping sickness and there were no trypanosome free cases with counts of 10 or less in whom any symptoms of ill health could not be more readily accounted for by some unconnected disease. So far as it went then the clinical findings supported the belief that cases with normal or only slightly raised counts were free from infection.

(ii) *Presence or absence of trypanosomes* In all the patients examined trypanosomes were only found in gland juice in two cases in blood film in one and in the C.S.F. in one. Since many more than this number were certainly still suffering from sleeping sickness this criterion also proved valueless.

(iii) *C.S.F. picture* A cell count of the C.S.F. supported by estimation of total protein, was considered the only reliable criterion of cure in survivors. Figures only slightly above the normal gave rise to some difficulty which is discussed below.

(iv) *Mortality* A history of death from sleeping sickness could only be obtained by questioning relatives and friends of the deceased, and therefore lacked the objective value of C.S.F. examination. Nevertheless, its use as a criterion of failure is unavoidable and it is believed that the figures obtained are subject to only slight error. Every effort was made to obtain the truth. Village and section chiefs were questioned to corroborate the statements of relatives, and where possible the questionnaire was repeated after some weeks or months to see if the same answers were given. This was invariably found to be the case. A decision as to whether death had resulted from sleeping sickness or some unrelated cause was not so difficult as might be imagined in dealing with a primitive people since they were only too familiar with the late symptoms of sleeping sickness, and in many cases the cause of death was given with assurance as being due either to sleeping sickness or to one of a number of unrelated accidents or diseases such as a fall from a palm tree, pneumonia, strangulated hernia, or stricture. Where the cause of death was unknown or in doubt, it has been classified as due to sleeping sickness. As a check against deaths due primarily to sleeping sickness having been credited to other causes, the death rate from other causes has been worked out. The figures for patients who had received Courses 1 to vi are found to be 18, 15, 13, 25, 12 and 0 per thousand per annum respectively which is almost certainly

an under-estimate, and while 181 deaths were attributed to sleeping sickness or the toxic effects of the drugs employed, only 48 were attributed to other causes.

### INTERPRETATION OF CEREBROSPINAL FLUID FINDINGS

Four questions arise in deciding on the significance of the C.S.F. picture as an indication of cure. (i) Do any diseases coexist which might affect the picture? (ii) What is the relative significance of a raised cell count and of increased protein? (iii) Is it possible that, once the central nervous system has been seriously affected by sleeping sickness the membranes may remain permanently damaged so that the C.S.F. retains an abnormally high cell and protein content even though eradication of the infection has been achieved? If this were so a slight increase of these elements would not necessarily carry any pathological significance. (iv) What period must elapse after cure before the cell count reaches a final stable level? These questions will now be discussed seriatim.

(i) Coexistent syphilis and yaws are probably the only diseases which might theoretically have interfered with the interpretation of the C.S.F. findings. Fortunately syphilis is definitely rare in the community studied, and the writer remembers having seen only one definite case of primary or secondary syphilis, though he has seen countless numbers of cases with gonorrhoea, which appears to be almost universal among adults and chancroid and lymphogranuloma inguinale are not uncommon. Yaws, on the other hand is endemic—a causal connection probably exists between the prevalence of yaws and the rarity of syphilis—and it is estimated that considerably more than half the entire community become infected at some time in their lives, most frequently in early childhood. Many of the sleeping sickness patients harboured yaws at the time of the enquiry, mostly latent or limited in its obvious manifestations to clavus and other irregularities of the soles of the feet. The possible influence of yaws on C.S.F. interpretation therefore becomes important to consider.

As a minor check, a cell count was carried out on twenty people in the area who as far as could be ascertained, had never had sleeping sickness. Nineteen gave counts of 5 cells or less but the remaining one showed a count of 6½ cells by the routine method used for all patients. It is not known whether this person harboured yaws infection or not, but the result does indicate that a few of the slightly raised counts in the sleeping sickness patients may be due to chance variation in counting only 3 c.mm. or to some cause other than sleeping sickness.

Turning to the literature VAN DER SCHEER (1936) SLAMET SUDIPTO (1939) and GUTIERREZ *et al* (1942) have published C.S.F. cellular counts in yaws cases. These observers differ somewhat in the results they obtained at different stages of the disease, but if their total figures for all stages are combined it is

found that of 342 cases examined nine or 2.6 per cent. gave counts of 6 to 10 cells inclusive, and the same number counts of over 10 cells. GUTIERREZ *et al* also estimated the total protein in fifty-three cases and found that in none did the quantity exceed 35 mg per 100 c.c. though in ten or 18.9 per cent it fell between 30 and 35 mg. If the same figures apply in Sierra Leone the final sleeping sickness cure rate obtained in the present enquiry will tend to be a slight under-estimate.

(ii) Of the Sierra Leone cases whose CSF was examined at the final survey many more showed a count of above 5 cells than a total protein exceed ing 35 mg. and though there were a considerable number with a raised count combined with a normal protein (cp Cases 4, 6 and 7 in Table I) the converse was relatively rare. Thus in 917 fluids examined for both cells and protein, seventy-one were definitely abnormal (cells above 10 or protein above 35 mg or both) but of these only seven had a protein of over 35 mg combined with a cell count of 10 or less. The omission of protein estimation would therefore have entailed an error of about 10 per cent. in recording abnormal fluids, but in calculating total failures after treatment, including the dead and the blind, the error would have been reduced to about 2 per cent. In preparing the tables of results which follow some arbitrary rule had to be adopted to include as failures the few cases who had a raised protein but a normal or only slightly raised cell count. For this purpose cases with a cell count of 5 or less but a protein of 31 to 35 mg have been included in the corresponding 5 to 10 cell group and cases with a cell count of 10 or less but a protein of over 35 mg in the corresponding 10 + group. In other words a protein of 31 to 35 mg is taken as having the same significance as a cell count of 5 to 10 and a protein of over 35 mg the same significance as a count of over 10.

It should be mentioned that some workers, particularly the French, place more reliance on the total protein figure than on the cell count in the CSF and there also seems to be some divergence in opinion as to what is to be regarded as the upper limit of normal for protein. All one can say is that in Sierra Leone the cell count appeared to be the more sensitive indication of cure.

(iii) To shed light on the question whether the CSF picture may never return quite to normal in some cases, even though permanently cured a number of patients were subjected to repeated lumbar puncture at intervals. In Table I forty five of these cases are tabulated. They are not a random sample but are selected from some scores of cases to illustrate a variety of trends. The writer owes the majority of the figures for counts up to 6 months to Dr E. M. LOURIE. This table together with other figures not here tabulated shows clearly that a slight increase of cells may persist for at least 3 years in a proportion of cases particularly among those who showed marked alteration of the CSF before treatment, though there is every indication that treatment has been successful. Cases 13, 16, 36, 42, 43 and 45 illustrate this trend. In the three

last mentioned cases particularly it seems most improbable that the cell count would not have risen above 12 in 3 or 4 years had treatment not effected a cure. Clinically also these patients appeared perfectly well.

(iv) Table I also provides some information on the period which must elapse before the cell count reaches a final stable level. It shows that C.S.F. examination up to 9 months is of only limited value in assessing cure. At 4 to 6 months, not only are there many cases with a considerably raised count which are destined eventually to come down to normal without further treatment (cp Cases 1 3, 9 and 23) but cases actually occur who have a normal count at 4 to 6 months but are evidently not cured, since their count rises again later to grossly abnormal levels (cp Cases 6 7 14 15 and 24). The first tendency is more common after courses containing trypanamide, and the latter after the diamidines but whatever drug has been employed either event can occur. At 7 to 9 months a definitely raised count is of greater significance but there is still no certainty—borderline counts may be on their way up (Case 29), or down (Case 27) and even a markedly abnormal count of 24 may not signify failure (Case 32). Unfortunately no cases were examined at 10 to 12 months. Of the six cases (Cases 2 and 33 to 37) lumbar punctured at 13 to 15 months, three with borderline counts have come down to normal after a further interval one (Case 35) has gone up significantly and is probably still infected, while Cases 2 and 36 have gone up slightly but are probably cured, with a count which will continue indefinitely to fluctuate around or slightly above the normal. Of the seven borderline cases examined at 16 to 18 months and re-examined later four have returned to normal, two have remained stationary and one has increased slightly but probably not significantly from 10 cells to 18.

From a consideration of these and other figures the writer feels that it is impossible to form a sound estimate of cure within 9 months of treatment, that it is not entirely satisfactory to attempt one at 13 to 15 months and that it is preferable to wait until after 18 months. By this time there will be few uncured cases whose cell counts will not have risen considerably above normal, and a figure of about 20 cells will then serve to differentiate cured cases with a permanent slight excess of cells from uncured cases with a rising count. In the present study the average interval was about 17 months but some cases were re-examined as early as 13 months after treatment. For this reason, more stringent figure of 10 cells has been taken as the dividing line.

## RESULTS

### PART I.—PROTECTIVE EFFECT OF TREATMENT AGAINST SUBSEQUENT REINFECTION

While the relapse rate in previously treated cases was under investigation an interesting sidelight was thrown on the protection conferred by treatment against the subsequent reappearance of trypanosomes in the body fluids. Not

TABLE I.

REPEATED C.S.F. CELL COUNTS IMMEDIATELY PRECEDING, AND AT VARIOUS INTERVALS AFTER TREATMENT

No.	Drug.	Initial Count.	Months after start of treatment.					
			4-6	7-9	12-15	16-18	19-24	Over 2 years.
1	Pentamidine	23	16		2 25			
2	Tryparamide × 10	34	5		7 20			
3	"	52	22		2 28			
4	Pentamidine	148	13		33 25			
5	"	536	13		3 33			
6	A3 + T5	6	1			105 20		
7	Pentamidine	42	3			37 23		
8	"	166	17			130 50		
9	"	245	16			1 22		
10	Tryparamide × 10	300	10			1 20		
11	"	390	11			12 25		
12	A3 + T5	5	8				3 20	
13	Pentamidine	5	29				10 23	
14	"	10	3				58 60	
15	"	16	1				103 25	
16	"	21	8				8 25	
17	A3 + T5	29	8				3 20	
18	"	34	4				12 25	
19	"	52	12				0 23	
20	Propamidine	74	6				5 25	
21	"	98	3				22 30	
22	A3 + T5	110	3				20 20	
23	"	125	93				1 20	
24	Propamidine	225	3				205 60	
25	A3 + T5	1 400	18				3 20	
26	Tryparamide × 10	23	19		7 20			11 (31 m.)
27	A1 + T9	1		8 23	3 20			
28	A3 + T5	4		5 23	2 23			
29	A1 + T9	14		6 31	12 22			
30	A3 + T5	180		5 30	46 25			
31	"	355		5 50	2 23			
32	"	5		24			1 20	
33	A3 + T5	3			7 20			3 (37 m.)
34	Tryparamide × 5	4			6			2 (25 m.)
35	"	5			6			20 (25 m.)
36	T5 + Pentamidine 5	124			1			6 (25 m.)
37	"	250			"			2 (25 m.)
38	A3 + T5	1				8 20		2 (45 m.)
39	Tryparamide × 10	1-				9 22		3 (31 m.)
40	"	—				12 20		4 (45 m.)
41	A3 + T5	—				10 1		1 (44 m.)
42	"	—				8		8 (44 m.)
43	Tryparamide × 10	—				12 20		12 (45 m.)
44	"	—				10 25		18 (44 m.)
45	A3 + T5	4					10 20	9 (36 m.)

NOTE: 2 25 represents 2 cells, 25 rag. protein, etc.; 11 (31 m.) represents 11 cells 31 months after treatment, etc.

only was the number of cases who revealed trypanosomes in the peripheral circulation on re-examination a mere fraction of the number of obvious clinical and C.S.F. relapses but it was also very much less than the number of reinfections to be expected during an average interval of 17 months in patients who had continued to reside in a highly endemic area. Actually only four out of 1732 cases re-examined revealed trypanosomes—one in blood film, two in gland juice and one in C.S.F. (No special methods were employed to reveal trypanosomes in the C.S.F., as their presence in this site is unimportant from the point of view of transmission. They were merely looked for in the course of carrying out cell counts, and the positive case is included here for the sake of completion in recording cases who showed trypanosomes in a body fluid.) It is impossible to prove with certainty whether these cases constituted relapses or reinfections, though there are reasons, discussed below for believing that one was in fact a relapse and the other three were reinfections. It will therefore be convenient to consider the implications first on the assumption that all four were relapses, and then on the assumption that all were reinfections. For this purpose it is necessary to show for comparison the number of relapses based on grounds other than the reappearance of trypanosomes, and also the number of reinfections which might have been expected if treatment had conferred no protection against reinfection.

In Table II the relevant figures have been set out, where they are grouped by treatment courses as follows. (a) Tryparsamide alone (Courses ii and ix) (b) antypol alone or in combination with tryparsamide (Courses i, iii and iv) (c) pentamidine or propamidine alone or pentamidine in combination with tryparsamide (Courses v, vi, vii and viii). C.S.F. relapses are here defined as cases with more than 10 cells per c.mm. in the C.S.F. Since not all the cases re-examined were lumbar punctured, the figures have been calculated on the percentage basis found to obtain in those who were. (Slightly more than one half of the cases re-examined were lumbar punctured.) The method employed in calculating the expected reinfections requires more detailed explanation.

With a few exceptions, the patients under review were originally diagnosed and treated during a mass survey of the whole population of the area concerned, and the population not treated was free from infection at that time as far as could be determined by the routine methods of gland juice and blood film examination employed. On completion of the present enquiry the area was again surveyed and a new infection rate of 4.9 per cent. was obtained by the same methods in the previously untreated population. It is possible however that a proportion of the incidence may be accounted for by cases who had been missed at the first survey and had survived, and by casual infected immigrants from the adjoining parts of Liberia and French Guinea. Without going into detail, one may say with confidence that, though the influence of these factors cannot be accurately assessed, it is most improbable that they accounted

for as much as half of the observed infections. For the present purpose however it is safer to over- than to under estimate their influence, and accordingly 2.5 per cent. may be taken as a conservative index of true new infections occurring in the untreated population during the period between the first and second surveys. A similar percentage of the cases who had been treated and cured might have been expected to have become reinfected and to have revealed trypanosomes on re-examination if neither their previous infection nor their treatment had provided any subsequent protection. In column 11 the numbers of expected reinfections are set out on this basis viz 2.5 per cent. of the cases re-examined and apparently cured of their original infections (i.e., with C S F cell counts of 10 or less).

TABLE II

PROTECTION CONFERRED BY TREATMENT AGAINST SUBSEQUENT REINFECTION AND BLOOD OR GLAND JUICE RELAPSE.

Course of treatment	Cases re-examined	C.S.F relapses (> 10 cells)	Expected reinfections.	Observed trypanosome-positive infections.
(a) Tryparsamide alone	337	34	8	3 (2 G J 1 B.F)
(b) Courses containing antrypol	1,211	68	29	1 (C.S.F)
(c) Courses containing diamidine	184	30	4	0

G J = Gland juice

B.F = Blood film

If we assume that all four trypanosome positive cases were relapses and not reinfections then after tryparsamide there were three such as against thirty four cases who had remained uncured by C S F standards but after courses containing antrypol or a diamidine there was only one C S F positive case and no blood or gland juice relapses as against a total of eighty-eight C S F failures. While it is admitted that more peripheral infections might have been revealed if special methods, such as blood culture or animal inoculation, had been employed, it is at any rate clear that all the courses used, but most certainly those containing antrypol or a diamidine had a powerful effect in rendering even the uncured cases non infective for a prolonged period.

If on the other hand we assume that all four cases were reinfections, even so antrypol and the diamidines are shown to have exerted a durable prophylactic action following cure for only one case treated by either of these drugs revealed



trypanosomes, and that in the C.S.F. whereas thirty three might have been expected to do so if no protection had been afforded. In the case of antypol the evidence appears conclusive in that of the diamidines, where the numbers at risk were fewer it can only be considered suggestive. The possibility that some cryptic infections unrevealed by the methods used, may have occurred owing to the drugs having exerted a suppressive rather than a true prophylactic action, cannot be excluded even so the absence of demonstrable trypanosomes in the peripheral circulation would render such infections relatively unimportant from the point of view of transmission by tsetse.

The reasons for believing that three of the trypanosome positive cases were in fact reinfections and one a relapse are as follows. The three patients with positive gland juice or blood film were all clinically early cases when re-examined with large soft glands suggestive of recent infection, and all had C.S.F. counts of less than 10 cells with a protein of under 30 mg. It is unlikely that their C.S.F. would have been approximately normal if they had retained the original infections for which they had been treated over 15 months previously. By contrast the patient with trypanosomes in the C.S.F. was clinically a late case similar in appearance to other obviously uncured cases, and he showed a high C.S.F. cell count.

To sum up. If we accept these probabilities as correct, then trypanosomes were not demonstrable in the blood or gland juice of any of the 122 cases treated by a variety of courses who had relapsed or remained uncured on the basis of a raised C.S.F. cell count. Again, no reinfections could be demonstrated in 1,307 cases treated and apparently cured by courses containing antypol or a diamidine though thirty three might have been expected. On the other hand three reinfections occurred among 303 such cases after trypanamide alone, when eight might have been expected.

A tentative explanation offered as being consistent with these findings is regard to reinfection is that all the courses of treatment employed produced a temporary immunity due to the destruction of trypanosomes in circulation, while antypol and the diamidines (but not trypanamide) exerted in addition a prophylactic action persisting for over a year in the doses given (one or more doses of 1 gramme of antypol, five or more doses of 50 to 100 mg. of a diamidine). The prophylactic effect of antypol is of course already well known, while evidence that the diamidines exert a similar effect has been published by VAN HOOFF *et al* (1944) and by FULTON (1944). The reason for considering their effect here is to stress the value they may have in the control of an epidemic when used in combination with other drugs for treatment, apart from their immediate curative value.

However it is not claimed that the above results necessarily apply in all parts of West Africa. Probably a great deal depends on the local strain of trypanosome. As regards the reappearance of trypanosomes in the peripheral circulation in relapse after treatment, the writer previously found this to have

occurred in a large proportion of relapses in one small area in Nigeria after trypanamide alone, but in general his experience has been that it is rare in relapses after combinations containing antrypol. As regards prophylaxis he found in some experiments with clean guineapigs, to which he gave prophylactic antrypol and then attempted to infect at varying intervals afterwards by some Nigerian strains of recent human origin, that the duration of protection appeared to be influenced greatly by the strain tested as well as by its previous animal passages. The use of the syringe for transmission in these experiments however instead of the fly, precludes dogmatism. Yet again, in another part of Sierra Leone where an unusual type of the disease exists, about 9 per cent, of a group of cases revealed trypanosomes 18 months after treatment with a combination of antrypol and trypanamide, but whether they represented relapses or reinfections was not determined. After such variations have been taken into account, it probably remains true to say that, in most types of West African trypanosomiasis, the inclusion of antrypol or of a diamidine in treatment courses reduces danger to the community arising from the possibility of subsequent infectious relapse or reinfection among the cases treated.

## PART II—COMPARATIVE CURE RATES AFTER COURSES I TO VI

### *Mortality*

The most noteworthy feature relating to mortality within 3 months is the high toxicity of Course III compared with the other courses combining antrypol and trypanamide. LOURIE (1943) has already described the symptoms in these cases. There are very few deaths between 3 and 12 months after any course among cases whose initial count was 100 or less, but in cases with counts of over 100 the comparative failure of the diamidines is very evident. (Tables III and IIIA, pp 112 and 113)

### CEREBROSPINAL FLUID FAILURES

In Tables IV (p 114) and IVA the C S F results are set out in similar fashion. For the reasons already discussed, the writer considers a figure of 10 cells to be a fair criterion in separating successes from failures but the percentages of cases with counts above 5 are also given to satisfy the most exacting standards. Here again the most evident feature is the failure of the diamidines in late cases and there is a suggestion of inferiority even in the group with between 6 and 20 cells before treatment.

### IMPAIRMENT OF VISION

A patient who is cured of his infection but rendered blind in the process must be reckoned a failure and it is necessary to take visual impairment into account in computing successful results. Table V shows the percentage of cases whose vision was affected after different courses of treatment. The most notable feature is the high incidence of blindness (5.6 per cent.) in cases treated by trypanamide, though the figure is somewhat weighted by the high

TABLE III

DEATHS WITHIN 3 AND 12 MONTHS AFTER START OF TREATMENT CLASSIFIED BY INITIAL C.S.F. COUNT AND COURSE EMPLOYED, EXPRESSED AS PERCENTAGES OF CASES TREATED.

		(i)	(ii)	(iii) An- trypol 2 < 1 grammes trypan- amide 5 3 grammes	(iv) A + T other com- binations	(v) Pentam- idine	(vi) Propam- idine
C.S.F. Cell Count before Treatment	Cases (actual)	139	1	223	93	85	18
	Died < 3/1	0.7	—	5.8	* 2	—	—
	Died 3-12 m.	—	—	—	—	—	—
	Total deaths	0.7	—	5.8	—	—	—
	Cases (actual)		61	111	80	39	12
	Died < 3/12		1.6	9.1	1.7	8.1	—
	Died 3-1 m.		—	1.8	—	—	—
	Total deaths		1.6	9.9	1.7	8.1	—
	Cases (actual)		31	30	10	22	8
	Died < 3/12		3	3.2	—	9.1	—
	Died 3-1 m.		—	—	—	13.6	—
	Total deaths		3.2	3.2	—	22.7	—
	Cases (actual)		31	56	16	21	9
	Died < 3/1		6.3	8.6	1.3	9.7	—
	Died 3-12 m.		6.5	4.0	6.3	33.8	37.5
	Total deaths		12.8	12.6	18.8	43.2	37.5

Table III shows the mortality in cases classified by the C.S.F. cell count before treatment and the course employed, while Table IIIA condenses the results into two groups with pre treatment counts of above and below twenty cells respectively and incorporates also the cases not lumbar punctured before treatment. Deaths within 3 months have been shown separately because such deaths are attributable chiefly to the toxicity of the drugs employed, though a few occurred in patients who were moribund at the start and died before treatment had time to influence the disease. The remaining deaths tabulated are restricted to those occurring within 12 months in order to provide a fair comparison between different batches of patients followed up at anything between 12 and 28 months after treatment. Mortality at under 3 months, therefore reflects the toxicity of the drugs employed, while mortality at 3 to 12 months reflects failure to prevent a fatal outcome of the infection itself. Deaths known to have been due to causes unconnected with toxicity or trypanosomiasis are excluded.

TABLE IIIA

DEATHS GROUPED BY INITIAL C.S.F. CELL COUNTS OF (A) 0-20 AND (B) OVER 20) CONDENSED FROM TABLE III) TOGETHER WITH (C) A GROUP NOT LUSIBLY PUNCTURED PRIOR TO TREATMENT

			(ii)	(iii)	(iv)	(v)	(vi)
			Trypara- amide 10 x 2 grammes	Antrypol 2 x 1 grammes trypara- amide 5 x 2 grammes	A + T other com- binations	Pentam- idine	Propam- idine
C.S.F. Cell Count before Treatment	0-20	Cases (actual)	73	226	183	124	27
		Died < 3/12 %	1.4	6.5	2.5	1.6	—
		Died 3-12 m. %	—	0.6	—	—	—
		Total deaths %	1.4	7.1	2.5	1.6	—
	Over 20	Cases (actual)	62	80	26	63	14
		Died < 3/12 %	4.8	5.0	7.7	9.4	—
		Died 3-12 m. %	3.2	2.5	3.8	26.4	21.4
		Total deaths %	8.0	7.5	11.5	35.8	21.4
	No count	Cases (actual)	242	915	256	15	14
		Died < 3/12 %	2.6	6.6	1.6	20	7.1
		Died 3-12 m. %	1.2	1.1		6.7	21.3
		Total deaths %	3.8	7.7	1.6	26.7	28.3

proportion of late cases treated by this drug since vision is more frequently impaired by tryparamide in late than in early cases. The corrected figure, explained below and shown in Table VIII (p 118) is 4.8 per cent.

#### HEALTHY SURVIVAL RATE.

Healthy survivors may be defined as patients who were alive at the time of the survey were not blind, and had a C.S.F. count of 10 cells or less (or where indicated by the context, of 5 cells or less). For reasons already given, clinical condition is not here taken into account, but it may be accepted that no survivors with counts of 10 cells or less were clinically suspect. From the preceding tables a general idea may be gained of the healthy survival rate among cases treated at different stages of the disease by different courses. In many of the categories however the cases are too few for statistical purposes, the proportion of advanced and early cases (as revealed by the C.S.F. cell count before treatment) is not the same in the different treatment groups, and

TABLE IV

C.S.F. CELL COUNTS BEFORE AND MORE THAN A YEAR AFTER TREATMENT  
RESULTS EXPRESSED AS PERCENTAGE OF CASES RE-EXAMINED BY LUMBAR PUNCTURE.

C.S.F. before treat- ment	C.S.F. after treatment	(i) Antrypol 5 x 1 grammes	(ii) Trypara- mide 10 x 4 grammes	(iii) Antrypol 3 x 1 grammes, trypara- mide 5 x 2 grammes	(iv) A + T other com- binations	(v) Pentam- idine	(vi) Propam- idine
0-5 cells	Cases (actual)	47	5	110	26	89	8
	0-5 cells %	85.1	80	96.4	96.2	91.5	100
	5-10 cells %	10.6	20	0.9	3.8	3.4	—
	10 + %	4.3	—	2.7	—	5.1	—
	Total 5 + %	14.9	20	3.6	3.8	8.5	—
6-20 cells	Cases (actual)		27	47	36	24	8
	0-5 cells %		74.1	89.4	80.6	78.0	83.3
	5-10 cells %		14.8	8.5	16.7	8.3	—
	10 + %		11.1	2.1	2.8	16.7	16.7
	Total 5 + %		25.9	10.6	19.4	25.0	16.7
21-100 cells	Cases (actual)		11	20	7	11	8
	0-5 cells %		72.7	90.0	57.1	63.6	20.0
	5-10 cells %		27.2	5.0	28.6	9.1	20.0
	10 + %		—	5.0	14.3	27.3	80.0
	Total 5 + %		27.2	10.0	42.9	36.4	80.0
Over 100 cells	Cases (actual)		18	22	8	8	3
	0-5 cells %		62.5	68.6	75.0	12.5	—
	5-10 cells %		16.8	12.5	12.5	12.5	—
	10 + %		18.8	21.9	12.5	75.0	100
	Total 5 + %		37.8	34.4	25.0	87.5	100

not all the survivors have been lumbar punctured to show which were healthy and which were not. A means is therefore required for calculating what the percentage of healthy survivors after each course might have been if the proportion of advanced and early cases (i.e., the C.S.F. cell distribution) had been the same for each course, and if all, instead of rather less than one-half, of the survivors had been lumbar punctured. This percentage will hereafter be

TABLE IVa.

C.S.F. CELL COUNTS MORE THAN A YEAR AFTER TREATMENT GROUPED BY ORIGINAL COUNTS OF (a) 0-20 AND (b) OVER 20 (CONDENSED FROM TABLE IV) TOGETHER WITH (c) A GROUP NOT LUMBAR PUNCTURED PRIOR TO TREATMENT

C.S.F. before treatment	C.S.F. after treatment	(u) Trypara- mide 10 x 2 grammes	(ii) Antrypol 3 x 1 grammes trypara- mide 5 x 2 grammes	(iv) A + T other com- binations	(v) Pentam- idine	(vi) Propam- idine
0-20 cells	Cases (actual)	32	15	62	83	14
	0-5 cells %	75.0	94.3	87.1	86.8	92.8
	5-10 cells %	15.6	3.2	11.3	4.8	—
	10 + cells %	9.4	2.5	1.6	8.4	7.2
	Total 5 + %	25.0	5	12.9	13.2	7.2
Over 20 cells	Cases (actual)	27	52	18	19	8
	0-5 cells %	66.7	75.0	66.7	42.1	12.5
	5-10 cells %	22.2	9.6	20.0	10.5	12.5
	10 + cells %	11.1	15.4	13.3	47.4	75.0
	Total 5 + %	33.3	25.0	33.3	57.9	87.5
No count	Cases (actual)	93	203	78	7	2
	0-5 cells %	77.4	83.7	91.0	85.7	100
	5-10 cells %	11.8	10.3	6.4	—	—
	10 + cells %	10.8	5.9	2.6	14.3	—
	Total 5 + %	22.6	16.3	9.0	14.3	—

TABLE V  
PERMANENT VISUAL IMPAIRMENT

Course	Cases treated	Per cent. vision impaired	Per cent. blind	Total affected Per cent.
Tryparamide	454	1.8	5.6	7.3
A3 x 1 grammes + T5 x 2 grammes	1105	1.8	1.3	3.1
A + T other combinations	435	0.2	1.1	1.4
Pentamidine	181	0.6	—	0.6
Propamidine	42	2.4	2.4	4.8

NOTE. The figures in column two represent all cases treated less (1) incomplete course (2) untraced and (3) died.

referred to as the standardized cure rate. When such a means is found it will be possible to compare directly the results of the different treatment courses. The method which has been employed in this enquiry is similar in principle to that used in calculating standardized death-rates from crude death-rates.

The first step was to obtain the standard cell count distribution from the records of all patients, numbering 1 170 lumbar punctured before treatment this was found to be as follows 0 to 5 cells 52.2 per cent. 6 to 20 cells, 26.1 per cent. 21 to 100 cells, 9.1 per cent. 100 + cells, 12.5 per cent. This distribution may be taken as the normal for a random group of untreated sleeping sickness cases in this part of Sierra Leone at this time, so that the results of any particular treatment course after correction to this standard represent what is to be expected of that course in such a random group. It is to be noted that the cases not lumbar punctured before treatment were as far as is known entirely unselected and so constituted a random group. The chief reason why correction is necessary is that among the cases who were lumbar punctured some early cases were specially selected for treatment with antrypol only and some late cases for treatment with trypanamide only.

The further steps involved in calculating the standardized cure rate after each course are set out in Table VI in which, as an example, the cure rate after treatment with Course II is worked out. The figures in column (a) have been obtained from the case records. The other columns are self-explanatory.

TABLE VI

METHOD OF CALCULATING STANDARDIZED CURE RATE—COURSE II, TRYFAMAMIDE.

Cell count before treatment	Seeing survivors as % of cases treated	b % of survivors with 0-10 cells (from Tables IV & IVa)	c $\frac{x \cdot b}{100}$	Per 100 cases lumbar punctured before treatment	
				d Standard cell count distribution	$\frac{c \times d}{100}$ Healthy survivors
0-5	100	100	100	52.2	52.2
6-20	86.7	83.9	86.0	26.1	22.4
21-100	86.8	100	96.8	9.1	8.8
100 +	83.9	81.2	68.1	12.5	8.5
				Per 100 cases not lumbar punctured before treatment	
No count	80.2	80.2	79.6	100	79.6

Since there were 478 cases treated by trypanamide, of which 135 were lumbar punctured before treatment and 343 were not (see Table IIIA, p. 113) the standardized cure rate is —

$$\frac{(91.9 \times 135) + (79.6 \times 343)}{478} = 83.1$$

In Table VII are set out the standardized cure rates calculated in this manner for each of the treatment courses. Also included in this table are the rates obtained similarly but on the more stringent basis of a cell count of 0-5 as representing the limit of normality.

TABLE VII

STANDARDIZED CURE RATE OBTAINED BY DIFFERENT COURSES OF TREATMENT

Course	Cases (actual)	(a) % A/W C.S.F. 5 cells or less	(b) % A/W C.S.F. 10 cells or less
		A/W C.S.F. 5 cells or less	A/W C.S.F. 10 cells or less
ii. Trypanamide	478	70.3	83.1
iii. A3 + T5	1331	78.1	85.8
iv. A and T other combinations	435	85.7	93.5
v. Pentamidine	192	68.9	73.5
vi. Proparidone	55	71.6	73.1

(a) Percentage alive and well, i.e. not blind—with C.S.F. cell count of 5 or less.

(b) Percentage alive and well with C.S.F. cell count of 10 or less.

The most successful course is shown to be Course iv antropol plus trypanamide—other combinations, with a standardized cure rate of 93.5. The difference between this rate and that of Course iii, A3 plus T5 is statistically significant, while that between Course iii and Course ii trypanamide alone is not.

It is instructive to analyse the causes of failure after different courses of treatment in order to show how far failure is due to the toxic effects of the drugs employed and how far to lack of therapeutic efficiency. This has been done in Table VIII where deaths within 3 months of commencing treatment, and blindness provide a measure of toxicity while subsequent deaths and cell counts exceeding 10 indicate therapeutic failure. Course ii is seen to be accompanied by a high incidence of blindness and Course iii by a high death rate within 3 months while Course iv is satisfactory in both these respects. There is less divergence between Courses iii and iv when the figures representing therapeutic failure only viz. 6.7 per cent. and 4.0 per cent. are considered. This difference is not definitely significant and in theory no difference was to be expected.



It is more interesting to compare the index of therapeutic failure in both courses containing antrypol plus trypanamide (Courses iii and iv) combined with that for trypanamide alone. The figures are respectively 8.0 and 9.8 per cent. If the standard error of these percentages is based on all the cases treated by each course (i.e. the cases on which the death rate is calculated) it is found to be 1.3 and the difference of 3.8 is significant, but if it is based only on the cases lumbar punctured after treatment (i.e. the cases on which C.S.F. failures are calculated) it becomes 2.3 and the difference is not significant. There is thus no conclusive proof that a course containing a total of some eight doses of antrypol and trypanamide in combination was more effective in this part of Sierra Leone at this time than one containing approximately the same number of doses of trypanamide alone, though there is a suggestion that this is so. One might expect such a difference if some of the strains of infecting

TABLE VIII.

## CAUSES OF FAILURE AFTER TREATMENT

Figures represent percentages of total cases treated by each course. All figures are standardized.

Cause of failure	ii Trypan- amide × 10	iii Antrypol × 3 + trypan- amide × 5	iv A + T other com- binations	v Pentam- idine	vi Propam- idine
Died < 3/12	2.3	6.4	1.3	4.7	1.8
Blind	4.8	1.1	1.2	0.0	1.6
Died > 3/12 or C.S.F. < 10 cells	9.8	6.7	4.0	1.8	23.5
Total failures	16.9	14.2	6.5	26.5	26.9

trypanosome possessed a degree of trypanamide resistance. No proof has yet been obtained that trypanamide resistant trypanosomes occur in the area under investigation, but an occasional resistant case has come to light in other parts of Sierra Leone, and Colonel LE ROUZIC, who is in charge of the trypanosomiasis campaign in French Guinea, has informed the writer that such cases are by no means uncommon in parts of that territory which adjoin Kailabas District. All that can be said is that trypanamide resistance was uncommon in the area concerned, but in other territories where this feature is common a more marked superiority may be expected of a combined course over trypanamide alone.

Before concluding the review of these courses of treatment it is worth

referring again to the high degree of toxicity exhibited by Course III three doses of antrypol followed by five of trypanamide, all at 5-day intervals. Firstly as Table III shows no correlation existed between the stage of the disease (i.e. CSF cell count) and the probability of death within 3 months. One possibility investigated was the influence of riboflavin deficiency which was very much in evidence in the area at the time of treatment. Considerable numbers of sleeping sickness patients were carefully examined before treatment to reveal signs of this deficiency and patients with such signs were watched to see whether they tended to develop toxic symptoms during treatment more frequently than those without but this did not prove to be the case. Another hypothesis tested was that toxicity might be traceable to previously damaged kidneys on the supposition that antrypol would increase the damage and so delay the excretion of the trypanamide which followed, thus producing a cumulative arsenical poisoning. The urine of a number of patients was tested for albumin before treatment and the patients observed for toxic reactions but here again, though a considerable proportion of patients showed albumin no correlation was found to exist between this factor and toxicity and in the majority of cases albumin had disappeared by the end of the course. The mechanism of production of the toxic reactions was therefore not solved unfortunately postmortem examinations which might have elucidated it, were not possible. As regards the great reduction in toxicity which followed when the same course was given but an interval of over a month was allowed to elapse between the first and second doses of antrypol, the most likely explanation is that the interval allowed time for recovery in the patients general condition after the initial dose had destroyed all trypanosomes in the peripheral circulation. The course comprising two doses of antrypol followed by trypanamide all at 5-day intervals was found to be nearly as toxic as Course III and there seems no doubt that, of all the courses reviewed, the best combination in Sierra Leone is the one which allows an interval between the first and second antrypol but is otherwise the same as Course III. The non toxic value of this course was discovered by accident when an initial dose of antrypol was given immediately after diagnosis for the purpose of rendering the patient non-infectious to tsetse until treatment proper could be started, and this protection is an additional advantage where it is not possible to arrange for treatment to follow diagnosis immediately. A satisfactory rule for all occasions has now been worked out if it is convenient to arrange an interval of 3 weeks or more without treatment after the first dose of antrypol has been injected, then a further two of antrypol followed by five of trypanamide are given if the interval is between 2 and 3 weeks, only one further antrypol and six trypanamide are given and if the interval is less than 2 weeks, then no further antrypol is administered and the course is completed with seven doses of trypanamide. This rule has since been applied over many thousands of cases and it has been consistently found that no more than about 1 per cent. of patients die during treatment and no more than 1 per cent. become blind.

## PART III.—TREATMENT BY A COMBINATION OF PENTAMIDINE AND TRYPARSAMIDE ADMINISTERED CONCURRENTLY

Various authors writing on the treatment of sleeping sickness with pentamidine have stressed the advantage of a drug which can be given by daily injection. This may be true for the limited number of patients in Africa who live close to a hospital or dispensary and for the still more limited number for whom inpatient accommodation can be found, but it is certainly not true for the majority in West Africa who must still be treated by mobile teams at centres far from their homes. In the writer's experience it is generally less upsetting to the African peasant's farm work and normal life to visit a treatment centre once every 5 or 7 days than to visit it daily for the same number of times, and whereas he would not be willing to make a journey of more than 3 or 4 miles daily for perhaps 10 days, he will and does walk four times the distance regularly once in 5 days on ten occasions. This means that in mobile campaigns a much larger area can be brought under treatment at one time when injections are spaced, and such spacing allows an injection team to treat at other centres during the intervals. These factors more than compensate for the short period required to complete treatment in the individual by daily doses.

Greater practical advantage would therefore accrue from reducing the number of attendances necessary in the spaced type of course while retaining its effectiveness, than from reducing the intervals between injections. Such an aim might be realized by employing a combination of trypanamide with another drug such that the two drugs could be administered concurrently in full doses without undue toxicity. Antryptol as the second drug does not meet the need because, when given concurrently with trypanamide, the dose of one or both drugs must be substantially reduced for the combination to be tolerated. In the trial now to be described pentamidine was used, and five 100 mg. doses of this drug were combined with five 2 grammes doses of trypanamide, both drugs being given on each of five injection days at 5-day intervals so that the whole course was completed in 3 weeks. For comparison, in another group of cases pentamidine was given daily between the first and second doses of trypanamide, while in a third group used as a control, trypanamide only was given. It may be recalled that pentamidine, though ineffective by itself in late cases, has been shown to be equally as effective as antryptol in the first stage (cp. Tables III p. 112 and IV p. 114).

Eighty four cases were obtained and confirmed by the demonstration of trypanosomes in blood or gland juice, all were lumbar punctured, and they were then divided into three groups of similar cell distribution for treatment by Courses vii, viii and ix described in the introduction. Fifteen months later sixty three of the cases were obtained and re-examined, sixteen cases were not available though reported alive and well, four had died of unrelated causes and one, though apparently cured of his sleeping sickness, was suffering from a paraplegia which had commenced shortly after treatment with Course vii.

With the possible exception of this patient no toxic reactions occurred beyond the usual transient symptoms common immediately following pentamidine injections. No case died of sleeping sickness and none became blind.

The findings on re examination are summarized in Table IX. The control group of patients treated with five doses of trypanamide only were

TABLE IX

TREATMENT BY A COMBINATION OF PENTAMIDINE AND TRYPARAMIDE GIVEN CONCURRENTLY TOGETHER WITH A CONTROL GROUP TREATED BY 5 DOSES OF TRYPARAMIDE ONLY  
C.S.F. CELL COUNT BEFORE AND 15 MONTHS AFTER TREATMENT

		C.S.F. count after treatment	vii. Pentamidine 100 mg × 5 + T 2 grammes × 5 All at 5-day intervals	viii. Pentamidine 100 mg × 5 daily + T 2 grammes × 5 at 6-day intervals	ix. Trypanamide 2 grammes × 5	Total all treatments
C.S.F. Cell Count before Treatment	0-5	Cases	4	4	10	18
		0-5 cells	4	3	7	14
		5-10 cells	0	1	3	4
		10 + cells	0	0	0	0
	6-90	Cases	8	5	5	19
		0-5 cells	8	5	6	19
		5-10 cells	0	0	0	0
		10 + cells	0	0	0	0
	≥ 100	Cases	2	3	3	8
		0-5 cells	1	3	3	7
		5-10 cells	1	0	0	1
		10 + cells	0	0	0	0
	100 +	Cases	6	3	4	13
		0-5 cells	5	3	2	10
		5-10 cells	0	3	1	4
		10 + cells	1	2	1	4
	All counts	Cases	20	20	23	63
		0-5 cells	18	14	18	50
		5-10 cells	1	4	4	9
		10 + cells	1 (5.0%)	2 (10.0%)	1 (4.3%)	4 (6.3%)
		Total 5 +	2 (10.0%)	6 (30.0%)	5 (21.7%)	13 (20.6%)

Cure rate as shown (a) 10 cells or less = 93.7 per cent. (b) 5 cells or less = 79.4 per cent.  
Corrected rate = 97.2 per cent. Corrected rate = 81.7 per cent.

found to have responded so unexpectedly well that definite evidence of superiority of the pentamidine—trypanamide combination could hardly be hoped for in so limited a series. In the event, no significant difference was to be observed between the three groups, and in the aggregate of sixty-three cases 93.7 per cent. revealed a C.S.F. count of 10 cells or less and may be considered cured. The series contained a high proportion of late cases, including about half a dozen who were quite helpless or demented, and when the results are standardized for comparison with Courses 1 to VI the corrected cure rate rises to 97.2 per cent. Lack of toxic reactions and of blindness is largely responsible for the comparatively high rate. Estimations of total protein in the C.S.F. were not done in this series but, as has been already shown, any error arising from the omission is likely to have been very slight.

### DISCUSSION

There is no doubt that a great variety of strains of human trypanosome exist in different parts of West Africa, and that corresponding differences occur in the response of sleeping sickness to treatment. LESTER (1933) isolated from cases of the disease in Nigeria a number of strains whose behaviour ranged from that usually considered characteristic of *T. rhodesense* to that characteristic of *T. gambiense*. The present writer when working in Nigeria became acquainted with considerable variety in response to treatment in different areas. In one area some 500 cases were followed up about 18 months after they had been treated by a course of up to fifteen 2-gramme doses of trypanamide, most patients having received the full, or nearly the full, course in striking contrast to the present results in Sierra Leone nearly 50 per cent. were found either to have died or to have suffered blood or gland juice relapse. The Nigerian standard course of three doses of antypol followed by five of trypanamide was then substituted and all people found infected in the area both new infections and relapses, were treated with this combination. A year or so later very few cases could be found. In many other areas a single mass treatment with antypol and trypanamide brought a high infection rate down at one stroke and for a long period to a mere fraction of its former figure. Nevertheless in at any rate one area, in the neighbourhood of Gadamu, the writer found clinical and C.S.F. (cell count) relapses to be not infrequent after the standard course, though even here, as in Sierra Leone peripheral trypanosomes were scarcely ever found in such relapsed cases. A human strain was isolated in this area at this time which withstood dilutions of reduced trypanamide *in vitro* at 37° C. down to 1:1,000,000 for 24 hours, and after a few animal passages succumbed to normal human serum in 3 hours. It developed a fair degree of virulence for laboratory animals. Instances could be multiplied, but enough has been said to illustrate the argument that the response to a given course of treatment may be expected to differ considerably from place to place.

The average run of cases in Sierra Leone responds very readily to treatment, at any rate with tryparsamide and to this extent does not provide the best medium for testing new drugs and combinations, for which purpose a more resistant medium would show up differences more clearly. Nevertheless it seems probable that the response in different localities varies more widely to tryparsamide, owing to different degrees of natural or acquired tryparsamide resistance on the part of the infecting organism than it does to antrypol or pentamidine, so that results obtained with these two latter drugs may be expected to be of greater general applicability. Unfortunately antrypol and pentamidine are of little value alone in cases with serious involvement of the central nervous system, for which tryparsamide probably remains the most effective single drug. For large scale treatment therefore when regular C.S.F. examinations may not be possible a combination of tryparsamide with another drug is indicated. If further trials confirm that the concurrent administration of pentamidine with tryparsamide is free from danger, then this combination may well prove the most generally useful, since it carries the great practical advantage of reducing the number of attendances and the duration of treatment required by a combination whose components can only be administered in succession. The property possessed by antrypol of reducing the risk of reinfection or blood relapse for some months after treatment is apparently also shared by pentamidine. There is one drawback to the administration of pentamidine in the form of the hydrochloride, viz. the immediate fall of blood pressure with attendant symptoms which it often produces, and this feature might well frighten certain classes of patient and preclude its use by unsupervised African injectors but it is claimed that the substitution of the much more soluble methionate, of which the writer has as yet had no experience, makes it possible to give the drug in no more than 1 or 2 c.c. of distilled water by the intramuscular route, thereby reducing the rate of absorption and so eliminating this unpleasant side-effect.\*

To conclude, the possible advantages for mass treatment of a combination of pentamidine and tryparsamide administered concurrently would appear to justify a more extended trial in a locality where sleeping sickness shows a less ready response to tryparsamide alone than is the case in Sierra Leone.

#### SUMMARY

1 The results are described of an individual survey in Sierra Leone of over 2,000 sleeping sickness patients who had been treated by various courses of drugs more than a year previously, 1,800 of the patients were seen and examined and in nearly 1,000 of them examination of the cerebrospinal fluid was carried out.

\* The writer has since used pentamidine methionate in 150 mg. doses intramuscularly in some 200 cases in a prophylactic trial, and has found that the unpleasant side-effects of the hydrochloride are in fact eliminated when the methionate is used in this manner.

2. Criteria of final cure are discussed, and it is shown that a decision in individual cases cannot be arrived at with certainty within about 15 months after treatment. The only criteria accepted in this survey are (1) reliable information regarding mortality and (2) the observed C.S.F. findings more than a year after treatment. Patients rendered blind by treatment were reckoned as failures.

3. The C.S.F. cell count is shown to be a more sensitive indication of failure in Sierra Leone than total protein estimation. In a minority of cases the cell count is found to remain slightly raised up to 3 or 4 years after treatment even though there is every indication that cure has been complete. For this reason a figure of 10 cells has been accepted as the limit of normality in assessing the cure rate, but to satisfy the most stringent standards, the percentages of cases with final counts below and above 5 have been furnished in addition.

4. Since late cases are harder to cure than early ones, and since the proportion of late cases differed among the groups of patients treated by different drugs, the final results have been corrected to show the expected cure rate after each course in a collection of cases with standard C.S.F. cell distribution before treatment. The best combination of antryptol and trypanamide is shown to yield a corrected final cure rate of 93.5 per cent.

5. An incidental finding has been the prolonged protection against overt reinfection conferred by antryptol and probably also by pentamidine.

6. In a small scale trial with a course which combined pentamidine and trypanamide given concurrently in full dosage and was complete in 3 weeks, results were very promising though the average Sierra Leone patient reacts so well to trypanamide alone that it was impossible to prove the superiority of the combination. The risk of toxic sequelae and visual impairment with this combination appears to be very slight, and it is recommended for more extended trial in other parts of West Africa where the average case is more refractory to treatment with trypanamide.

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## LEPROSY OF THE LARGE INTESTINE AND GALL-BLADDER. CASE REPORT WITH REVIEW OF THE LITERATURE.

BY

CARL E. TAYLOR, M.D.\*

*From the Board of Health Laboratory, Gorgas Hospital, Ancon, Panama Canal Zone*

The gastro-intestinal and biliary tracts are among the few portions of the body which are generally supposed to escape involvement in leprosy. The purpose of this paper is to report a case of leprosy colitis and cholecystitis.

### REVIEW OF THE LITERATURE.

Tuberculosis is so often encountered in cases of leprosy that pathological lesions of the gastro-intestinal tract have usually been attributed to tuberculosis. This observation was made by HANSEN and LOOF (1894) at the very outset of the definitive study of leprosy; they believed that there was no such thing as pulmonary or intestinal leprosy. Ever since then any deviation from this concept has been regarded with scepticism. FRITZ (1943) in an authoritative review of the pathology of leprosy stated that in the early years of the study of the disease a few cases were reported as intestinal leprosy but he thought they might have been misdiagnosed cases of tuberculosis. This is probably true of most of the reported cases such as those which DANIELSEN and BOECK (1848) described as resembling tuberculosis but a few of the reports deserve more careful attention.

The case description that seems to be most authentic was published by Von REISNER (1896). He was fully aware of HANSEN and LOOF's observations and had personally studied some of their material. In his own series of seventeen autopsies on lepers in Riga, he found two that had typical intestinal and pulmonary tuberculosis. His other case with intestinal lesions was a 70-year-old man with extensive cutaneous and lymphatic leprosy. A few pleural adhesions were the only pathological changes in the lungs; there was no evidence of tuberculosis. He found many shallow ulcers with undermined borders in the colon and a few in the ileum. There was round cell reaction but he could find no giant cells or caseation necrosis. Many acid fast bacilli were present in these ulcers, both

\* The author is indebted to Captain B. H. KEAN M.C., A.U.S. and Major WRAY J. TOMLINSON M.C., A.U.S. for assistance in the preparation of this paper.



similarity and in the large clusters considered typical of leprosy. These bacilli showed the relatively rapid decolorization of basic stains by acids which even then was recognized as being a valuable criterion for the differentiation of the acid-fast bacilli. With this much evidence Von Ruzicka could claim that this was intestinal leprosy almost as positively as we can say even today that any lesion has been caused by leprosy bacilli.

SCHULTZ (1903), in reviewing the various pathological manifestations of leprosy made the following statement: "As to the intestine, DOETSCHLE and WOLFF in their case found spots under the muscularis mucosae, and SCHIFFER in one case discovered, close in front of Bauhin's valve a few somewhat raised whitish yellow areas with depressed centres which were found to consist of leprosy tissue. In this case there was also leprosy tissue of the mesenteric glands. CASTELLANI and CHALMERS (1915) stated, 'the typical lepromatous infiltration may occur in the submucosae of the intestine.' WASS (1926) observed that the gastro-intestinal tract is very infrequently involved in leprosy and the mesenteric lymph nodes only occasionally contain bacilli."

In 1918 MITSUDA (1936) published an article in Japanese which has since been reprinted in English. Using several fat stains, he claimed to have identified aggregations of lepra cells and occasional bacilli in many organs which previously had been thought to remain free from invasion. He said he found leprosy tissue in the oesophagus, stomach, intestine, mesenteric glands, pancreas, salivary glands, myocardium, lungs, bladder, prostate, uterus, ovaries and suprarenals. No indication of the frequency of occurrence of intestinal lesions is given except for the statement, "some of the changes here described are invariably seen in the organs of lepra nodosum." He summarized his findings with the following statement: "even the few advocates of intestinal leprosy have depended upon the microscopic examination of ulcers or ulcers and have demonstrated the existence of the leprosy bacillus in a case or two. It is no wonder that many scholars deny the existence of intestinal leprosy. They are evidently not aware of the fact that in the upper and lower parts of the muscularis mucosae of the stomach and intestine there usually develop numbers of lepra cells, and ultimately so extensive a pathological change as to be beyond comparison with that in any other viscera, while no change whatever is to be observed in the mucous membrane." MITSUDA concluded that Von Ruzicka's case was tuberculous solely because in his experience he had found, "absence of ulceration is a characteristic of intestinal leprosy and therefore those seldom if ever as any clinical disturbance." Except for the earlier observations made by CASTELLANI and CHALMERS, MITSUDA's findings have not been confirmed. I have studied sections, stained with a modified Ziehl-Neelsen stain using 5 per cent. sulphuric acid for decolorization, which were taken from various portions of the apparently normal intestines of two lepers. No bacilli were found.

There is an unusually high incidence of cholelithiasis among lepers. JEAN and CHILDRESS (1912) found an incidence of 11 per cent. in their series of 103 autopsies on the Isthmus of Panama. Perhaps this is associated with the hepatic lesions and the lipaemia which are frequently present in leprosy. No definite evidence of leprosy cholecystitis has been found in these cases of cholelithiasis. DANIELSEN and BORECK (1943) in their treatise on the pathology of leprosy described an infection of the gall bladder in which many superficial, whitish tubercles appeared on the mucosal surface. They suggested that this might lead to stagnation of the bile with the subsequent formation of calculi.

### CASE REPORT

The patient was a 58-year-old black bartender who had been born in Barbados, British West Indies, and had lived on the Isthmus of Panama since 1912. The family history was negative. The patient had never had typhoid, dysentery, malaria, syphilis, or any surgical procedures. The only known contact with leprosy was his having served beer to a leper on one occasion.

He was first seen at Gorgas Hospital on 4th July 1934. Five months before admission he had noticed small discrete papular and vesicular lesions

on his arms. Additional lesions then appeared on the cheeks forehead, ears, trunk, and lower extremities. He also experienced a slight loss of tactile sensibility. A review of systems was negative.

Physical examination at this time revealed papular lesions hyperkeratosis hyperpigmentation and diminution of tactile sensibility of the skin of the face and extremities. Small nodules were felt in the ears. There was pitting oedema of the ankles. Examination of the blood and urine was essentially negative. His blood Wassermann and Kahn reactions were negative on admission. During the following 6 years positive reactions to either one and occasionally to both of these tests were obtained but without any consistency. He received antiluetic treatment and after 1940 all tests were negative. All stool examinations were negative for amoebae and parasites. Throughout the course of his disease repeated examinations of smears from the nasal septum and various skin lesions were positive for *Mycobacterium leprae*.

When the diagnosis of leprosy was established he was transferred to Palo Seco Leper Colony. While there he received intensive treatment with the following drugs: chaulphosphate, neoarsphenamine sodium bismuth tartrate 0.5 per cent. iodine, crude chaulmoogra oil, neoarsphenic bismuth salicylate and diphtheria toxoid.

By July 1937 he had begun to show resorption of the phalanges of the hands and feet in addition to the cutaneous manifestations. This osseous resorption progressed and was quite marked by April 1939 when radiologic studies were performed. In 1940 the skin lesions spread extensively and he developed definite delusional ideas of persecution. In January, 1943 the skin lesions which had been maculo-papular showed spreading diffuse infiltration and nodule formation. Deep bleeding trophic and lepromatous ulcers appeared on the hands and feet. His general condition underwent rapid deterioration. On 1st March 1943 he developed moderately severe diarrhoea which persisted to the end 1 week later. Basal pulmonary rales and ankle oedema appeared and he gradually lapsed into a moribund state and died on 8th March, 1943.

#### AUTOPSY

A complete autopsy was performed 2 hours after death at the Board of Health Laboratory. The patient was emaciated and cachectic, weighing 105 lb and measuring 67 inches in length. The final anatomical diagnoses were: Cutaneous leprosy of nodular lepromatous type involving entire body including scalp and external genitalia with multiple lepromatous ulcers; leprosy of peripheral nerves with trophic ulcers of extremities; ulcerative colitis; leprosy; haemorrhagic necrosis of gall-bladder wall, leprosy; chronic passive congestion and bronchopneumonia of lower lobes of both lungs; hepatomegaly (2,000 grammes); multiple lepromata of liver; portal cirrhosis, slight splenomegaly (300 grammes); leprosy lymphadenitis; generalized lepromatous infiltration

of mouth, nose, pharynx, and epiglottis    main en griffe," right osseous resorption of phalanges of all extremities    leproma of abdominal sympathetic ganglion    erosion of oesophageal mucosa    dilated lacteals in duodenum    arterioneurosclerosis, slight    testicular fibrosis    leprous keratitis    cataract, left    left lateral coloboma    leprous iritis    gynecomastia    dental caries, severe    infestation with *Strongyloides stercoralis*

No significant gross or microscopic pathological changes were found in the brain, cranial nerves, Gasserian ganglia, pituitary, thyroid, heart, or prostate. Careful examination revealed no evidence of tuberculosis in the lungs or any other portion of the body.

The pathological findings in the gall-bladder and gastro-intestinal tract deserve detailed description.

### Gall-bladder

**Gross Examination**—Striking gross pathological changes were evident in the gall bladder (Plate, Fig 1). It measured  $11 \times 5 \times 3.5$  cm. and the serosal surface was smooth and marble white. The wall measured 0.2 cm. in thickness at the fundus and 1.4 cm. near the base. It was firm and had a pale hyalined appearance. The mucosal surface had an unusual mosaic structure which was formed by small, pale yellow patches lying in a bed of reddish-brown necrotic tissue 3 mm. in thickness. Within the lumen there were approximately 25 c.c. of thick, viscid, yellowish-brown bile but no calculi. The extra hepatic bile ducts were widely patent.

**Microscopic Examination**—The architecture of all portions of the wall had been obliterated by necrosis. The indefinite, residual collagenous framework was distorted by oedematous swelling. Moderate diffuse inflammatory reaction, consisting of both polymorphonuclear leucocytes and small round cells, was present. The serosa was smooth. In some areas the mucosal folds could be vaguely identified but the epithelial cells had lost their identity. The rest of the mucosa had been completely destroyed by submucosal haemorrhage, the erythrocytes being fairly well preserved. The mosaic appearance of the gross specimen was obviously due to these irregular haemorrhages. Sections stained by a modified Ziehl-Neelsen stain, using 5 per cent. sulphuric acid for decolorization, revealed the presence of a few acid fast bacilli resembling *M. leprae*, some of them being arranged in typical parallel clusters. Much more abundant were numerous acid-fast granules which ranged from about the size of a coccus to several times the diameter of an erythrocyte. In a few places a definite transition from bacilli to granules was traced (Fig 2), with some of the bacilli assuming a streptococcal appearance. These acid-fast granules were concentrated along blood vessels and in scar tissue and several aggregations of them were seen within endothelial cells and in macrophages (Fig 3).



FIG 1—Gall-bladder and Large Intestine.

Gall-bladder shows thickened wall and mosaic pattern of mucosa. The upper segment of large intestine is from the sigmoid and shows large ulcers while the lower is the caecum and shows small ulcers.

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### *Gastro-intestinal Tract*

*Gross Examination*—The oesophageal wall was slightly thickened and over the mucosa were scattered confluent, serpiginous areas of erosion. The gastric rugae were hypertrophied and slightly inflamed. A few peritoneal adhesions were observed between loops of intestines. Projecting from the mucosal surface of the duodenum were six soft yellow pea-sized cystic masses. The small intestine was otherwise normal. The large intestine presented a truly remarkable appearance (Fig 1). From the ileocaecal valve to the anus the mucosal surface was studded with innumerable discrete greenish yellow nodules. These lesions were most numerous in the caecum but the largest ones were found in the transverse colon. A single lesion was found in the appendix. They ranged from 0.1 to 3 cm in diameter. The small lesions were in the submucosa but pointed upward through the mucosa. The larger nodules projected above the mucosal surface and showed superficial ulceration. The nodules were composed of greenish yellow, friable, necrotic material and surrounding some of them there were narrow haemorrhagic zones. In a few instances the ulcers extended almost to the serosa but no actual perforation was found. There was marked induration and oedematous swelling of the entire wall of the large intestine. The mesenteric lymph nodes appeared hyperplastic.

*Microscopic Examination*—The mucosa of the oesophagus showed shallow ulceration and slight inflammatory reaction of a non specific character. The duodenum was normal except for cystic papillomatous masses in the submucosa which appeared to be dilated lacteals. All portions of the wall of the large intestine showed moderate oedema. Most of the mucosal lining was fairly well preserved and contained only a few scattered small round cells and macrophages. The ulcers were composed of sharply demarcated masses of granular basophilic necrotic debris (Fig 4). Only a slight inflammatory reaction, consisting principally of macrophages was seen in and around these areas of necrosis. Some of the macrophages contained the typical vacuolated cytoplasm of lepra cells. No epithelioid cells or giant cells were present. The centres of the necrotic areas seemed to be in the submucosa with extension in both directions so as to ulcerate through the mucosa and almost penetrate the serosa. In sections stained by a modified Ziehl Neelsen stain using 5 per cent. sulphuric acid for decolorization scattered acid fast bacilli resembling *M. leprae* and a few typical parallel clusters of bacilli were found in the zones of oedema and inflammation around the margins of the areas of necrosis. (Fig 5). Within the necrotic masses a few acid fast granules similar to those seen in the gall bladder were recognized, together with many non acid fast bacilli and cocci of various shapes and sizes which appeared to be non specific representatives of the intestinal flora. A few small haemorrhages were seen adjacent to some of the centres of necrosis but most of the vessels had undergone thrombosis. No sclerosis or specific inflammatory reaction of the arterial walls

was recognized. The follicular architecture of the mesenteric lymph nodes had been obliterated by chronic, granulomatous inflammatory reaction which included many foamy lepra cells. No giant cells were seen.

### *Laboratory Findings*

Leprosy bacilli and globi were found in sections and smears from the following organs in addition to those found in the gallbladder and large intestine. In the epiglottis and nasopharynx there were many bacilli and globi. In the lepromata of the liver a moderate number of acid fast bacilli were seen. Scattered through the spleen there were a few bacilli. A few indistinct globi were found in the leproma in the abdominal sympathetic ganglion. In spite of the extensive lepromatous infiltration and cellular necrosis of the skin, no recognizable leprosy bacilli were found in sections or fresh smears. A few globi and indefinite acid fast structures having vague margins were identified in the skin sections. Fresh smears of the faeces contained larvae of *S. stercoralis*. Many smears from the margins of the intestinal ulcers were negative for amoebae and other parasites. Routine chemical studies of the blood were negative. Smears from the brain, spleen, rib marrow and blood were negative for malaria. No sickling was seen in a moist film of the blood. The specimens for bacteriological culture were lost.

### DISCUSSION

Among the immediate causes of death in leprosy various forms of dysentery and enterocolitis rank high. This was particularly true in Mexico where NÚÑEZ ANDRADE (1939) in 1 074 cases of leprosy found non specific enterocolitis leading all other causes of death with 15.5 per cent. He also reported that 0.6 per cent. of the deaths were due to amoebic dysentery. In Cullion Colony (PINEDA, 1924) the percentages were 1.3 and 3.8 respectively for enterocolitis and dysentery. In Panama (KEAN and CHILDRESS, 1942) enterocolitis caused 1 per cent. of the deaths in leprosy. It is important, then, to determine if the intestinal lesions in this patient might have had an aetiology other than leprosy. No amoebae were found after prolonged and careful search. The lack of bacteriological studies makes it impossible to state definitely that one of the pathogenic Gram-negative bacilli was not present. However the gross and microscopic pictures show no resemblance to any of the commonly recognized forms of bacterial colitis. Tuberculosis is ruled out by the morphological appearance of the bacilli by the whole histological picture and by the absence of tuberculosis elsewhere in the body. There was no evidence to indicate the presence of the rarer forms of enteritis.

The difficulties encountered in culturing and transmitting the leprosy bacillus make it impossible to incriminate it positively as being aetiological responsible for leprosy. In view of this fact only presumptive claims based on

the presence of the bacilli in lesions can be made. It is on this basis that this case is presented as being one of leprosy of the large intestine and gall-bladder.

The pathological picture of leprosy lesions in any portion of the body may show wide variations. Bacilli may be very numerous or completely absent in active lesions. The multiplicity of the systems of classification of leprosy points to the obvious fact that because of our present lack of information none of them is completely satisfactory. According to the Report of the First International Congress in Cairo (1938) classification of this case would be L N<sub>2</sub>. It is particularly difficult to classify definitely the pathological changes in the large intestine and gall bladder. There is nothing to suggest a tuberculoid reaction. The fact that both lepra cells and bacilli were present would suggest that these lesions belong somewhere in the lepromatous group. An intriguing theoretical possibility is suggested by the statements of MITSUDA (1936) and of CASTELLANI and CHALMERS (1913) that lepromatous infiltration of a relatively benign nature may occur in the intestinal submucosa. In this case the sharply localized necrosis may have occurred in areas of lepromatous infiltration. Since the patient died during a lepra reaction, the necrosis and the scarcity of bacilli may be attributed to the allergic factors which are thought to be responsible for lepra reactions. It may be pointed out also that the lepromatous infiltration of the skin showed extensive necrosis and ulceration in the presence of only a few globi while in the liver and epiglottis the bacilli were more numerous and the necrosis less marked. The difference in the degree of necrosis and conversely in the number of bacilli in these sites may be due partially to the presence of secondary infection in open ulcers of the skin and intestine as has been suggested by ERMAKOVA (1940).

Irregularly staining bacilli and granular acid fast bodies have been reported in cases of leprosy by HOFFMAN (1933) FAURE BEAULIEU and BRUN (1938) ERMAKOVA (1940) LOWE (1929) and others. In the last two of these reports a relationship between these forms and lepra reactions was demonstrated. ROGERS and MUIR (1940) outlined five distinct morphological variations which they observed during lepra reactions. They described rods containing a series of dots, giving the appearance of a string of beads and large, round, spore like forms. These are undoubtedly the same as the acid fast structures found in the gall bladder and intestinal lesions of this patient.

#### SUMMARY

The published discussions of intestinal leprosy are scanty and contradictory. There is some evidence for the belief that relatively benign leprosy infiltration of the submucosa of the gastro-intestinal tract may take place. It is possible that rarely, particularly during a lepra reaction, this infiltration may undergo necrosis causing active ulceration which is aggravated by secondary infection with intestinal flora. In the case which has been described, the patient died during an active lepra reaction and had diarrhoea terminally. Autopsy



revealed numerous necrotic ulcers along the entire large intestine there was also oedema, necrosis, and haemorrhage in the wall of the gallbladder. The presence of leprosy bacilli in these lesions and the absence of other aetiological agents provide presumptive proof that they were due to leprosy. As far as I have been able to determine, Von REINER is the only previously published case which can be considered to be ulcerative leprosy of the intestine. I have found no reports of leprosy cholecystitis.

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# THE CHEMOTHERAPY OF EXPERIMENTAL LEISHMANIASIS

## II A DOSE RESPONSE CURVE FOR THE ACTIVITY OF SODIUM STIBOGLUCONATE.

BY

L. G. GOODWIN B.Sc., B.Pharm (LOND)\*  
*The Wellcome Laboratories of Tropical Medicine London*

In Part I of this work (GOODWIN 1944) it has been shown that the number of parasites per 100 spleen cell nuclei of all types counted in spleen smears is a reliable measure of the degree of infection of Syrian hamsters infected with *Leishmania donovani*. It has also been shown that a single subcutaneous dose of an effective drug may have a measurable effect upon the parasite count in the spleen.

In the work reported in the present paper the above observations have been used to obtain a dose-response curve for the leishmanicidal activity of sodium stibogluconate.† The variation between the responses of individual animals and the conditions influencing the results have been investigated.

### METHODS

*Establishment and passage of strains of leishmania in the hamster*—Three strains of leishmania have been used in this work. Strain A (Indian kala azar) and strains L and H (Mediterranean kala azar isolated in culture in March, 1944). Strain A was transferred to hamsters by repeated intraperitoneal injections of cultures. Strains L and H were inocu-

\* The writer is grateful to Dr C. M. WENYON F.R.S., for his interest and encouragement, to Miss C. ROBERTS for checking the calculations and to Mr J. M. JUDG for invaluable technical assistance.

† Sodium antimony gluconate *Burroughs Wellcome & Co., Ltd*—Solustibosan™

lated into hamsters by the intrasplenic injection of primary cultures of human aternal puncture material. The intrasplenic route which was shown by ADLER and TCHERNOMORETZ (1941) to produce rapid and heavy infections, has proved to be a very useful method for the establishment in the hamster of freshly isolated strains.

Subsequent passages of all three strains were made by intraperitoneal injection of infected splenic material.

*Inoculation of test animals*—It is desirable that animals to be used in quantitative work should receive equal amounts of a standardized inoculum of infective material. It is not easy to standardize a suspension of hamster spleen but an approximately uniform inoculum was obtained by selecting a heavily infected animal (with a parasite count of 250 to 500 parasites per 100 spleen cell nuclei in biopsied spleen material) removing the spleen and emulsifying it in 25 ml. of sterile saline. The whole of the suspension was used to inoculate 50 fresh animals in as short a time as possible each animal receiving 0.5 ml. of the suspension intraperitoneally. Suspensions of large or very heavily infected spleens were further diluted. This method, although very approximate, has been found to give consistent results. Animals for inoculation were chosen to be as uniform in weight and in sex as possible, but as the supply of hamsters was limited it was not possible to exercise much selection of stock. However by recording the weights and sexes of the animals used, conclusions have been drawn as to the effects of these characters upon the results of leishmanicidal tests.

*Incubation period*—The degree of infection in the spleen of an animal suitable for the test is from 10 to 300 parasites per 100 spleen cell nuclei. Animals with counts of less than ten have been shown to be unsuitable for test, because a large number of nuclei must be counted to get a reliable figure (GOODWIN 1944). Heavily infected animals are sometimes resistant to drug treatment, and are very susceptible to secondary infections. Abscess formation in the pouches or in operation wounds, and sometimes oedema associated with kidney lesions have been observed (GOODWIN 1945). Animals with parasite counts of more than 300 are therefore best avoided. Between the limits of 10 and 300 parasites per 100 spleen cell nuclei hamsters are fairly uniform in response to drug treatment, as will be shown below and mortality from secondary infections is not heavy.

The length of time for the development of infection to this degree was found to depend more upon the strain of leishmania than upon the size of inoculum used. Strain A required 3 months (the incubation period found by most other workers), strain "L" required 6 weeks, and strain "H" only 3 to 4 weeks. Strain "A" is now in its twelfth, and strains "L" and "H" in their fifth hamster passages, and none has changed in virulence so far. Strain "H" is a useful test organism because of its unusually rapid development.

After a suitable incubation period, spleen biopsy and parasite counts upon a group of animals inoculated at the same time showed that 60 to 90 per cent. of them were suitable for use. The rest were either too heavily or too lightly infected.

*Technique of spleen biopsy*—The surgical removal of spleen fragments was performed under ether anaesthesia in the earlier experiments, but soluble hexobarbitone (100 mg/kg body weight given intraperitoneally) is now used. There is no danger of post-operative pneumonia with this anaesthetic and the animals require no attention on the operating table. The posterior end of the spleen was exposed with aseptic precautions, and a fragment 1 to 2 mm long removed with scissors. Bleeding rapidly ceased on application of a cotton pledget. The abdominal muscles were sewn together with fine rayon ligatures, and the skin secured with a running thread. Rayon caused fewer adhesions and less tissue reaction than catgut. The wound was swabbed with antiseptic and covered with collodion which prevented the animal from biting out the stitches until the wound had healed. Every animal was marked on the abdomen with a number by tattooing with Indian ink, and was isolated from other animals until the end of the test. Although the operation itself does not cause any deaths, animals infected with leishmania are susceptible to secondary infections, and failure to observe aseptic technique may lead to a fatal peritonitis or the formation of abscesses at the site of the operation.

*Estimation of degree of infection and the grouping of animals for test*—Spleen smears were prepared and counted by the methods described in Part I of this work. There was insufficient time between spleen biopsy and injection of drug to make full parasite counts so the smear from each animal was examined cursorily and classified as + (up to about 10) ++ (10 to 50) +++ (50 to 300) or ++++ (more than 300 parasites per 100 nuclei). The + and ++++ animals were rejected and the rest divided into groups as evenly as possible each group receiving both lightly and heavily infected animals. Care was also taken that the weights of the animals should be evenly distributed. Doses of drugs were injected on the day after spleen biopsy and the accurate parasite counts were made at leisure.

The spleens of infected animals are considerably larger than those of normal hamsters of the same age, and it was thought at first that the degree of infection at the initial biopsy might be roughly estimated from the size of the spleen. As it is difficult to make measurements during an aseptic operation a numbered series of outlines of typical spleen sizes was prepared, and is shown in Fig 1. The dimensions of these outlines are such that the area of each, measured with a planimeter is 1.5 times the area of the one before. Using this scale, the approximate spleen size of each animal operated upon was recorded both before and after drug treatment.

*Drug treatment and the assessment of effect*—Doses of the solution of sodium stibogluconate containing 2 per cent. of quinquivalent antimony used for

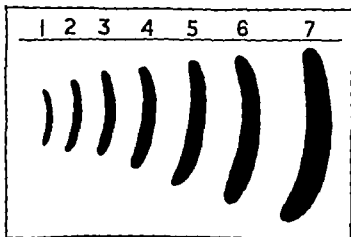


FIG. 1.—Standard set of outlines for comparison with the spleens of hamsters during operation (natural size). A normal adult hamster of weight 80 grammes has a spleen of size 3 to 4. In an animal infected with leishmaniasis the spleen is usually of size 4 to 7.

human therapy were injected under the loose skin of the backs of hamsters, care being taken to avoid injection into the pouches. The undesirability of starting treatment on the day after surgical operation is overshadowed by the fact that if left to recover for a longer period the degree of infection of the spleen increases rapidly. The parasite count before treatment would thus be rendered inaccurate. Because the abdomen had so recently been opened, doses were given subcutaneously not intraperitoneally.

One week after treatment, the animals were killed and a second set of spleen data prepared. Considerable care was exercised in the choice of 1 week as the best length of time for the drug to act before assessment of the effect. Repeated biopsies and counts were made at intervals after treatment and the results are discussed below.

## RESULTS.

### 1. *The dose-response curve*

Tables I and II show typical results of the treatment with sodium stibogluconate upon groups of hamsters infected with leishmanias strains A" and H.

Comparison of the parasite counts before and after treatment shows that large doses produced greater reductions in count than small doses, but that there was considerable variation between the animals at any one dose level. Some hamsters receiving small doses increased in infection but not so greatly as untreated control animals.

For the quantitative comparison of drugs, it is convenient if a finer relationship can be established between the dose and some function of the response. The results can then be treated by simple mathematical methods.

Dose (mg./Sb /kg.)	Weight (g.)	Spleen Size		Parasite Count per 100 nuclei.		% Reduc- tion	Mean % Reduc- tion	Ratio Before After	Mean Ratio Before After	Log Ratio Before After	Mean Log Ratio Before After ( $\bar{y}$ )	Standard Devia- tion ( $\sigma_y$ )
		Before Treat- ment	After Treat- ment	Before Treat- ment	After Treat- ment							
132	62	6	7	106	33	96.9	89.8	32	13.9	1.805	1.061	0.300
	65	6	8	97	10	89.7		0.7		0.087		
	74	7	7	60	51	91.6		12		1.079		
	69	6	6	42	4.9	88.6		8.8		0.014		
	97	6	6	37	2.6	92.0		14		1.146		
	65	7	6	34	1.6	95.6		23		1.363		
	65	7	6	34	0.1	73.2		3.7		0.569		
	95	7	7	33	7.1	77.6		4.5		0.653		
	107	6	6	50	2.3	95.4		22		1.312		
	76	6	7	22	1.6	92.7		11		1.116		
	125	6	7	16	2.6	83.8		6.2		0.792		
	100	6	6	16	1.2	89.4		0.1		0.072		
	116	6	6	14	0.5	96.4		29		1.447		
66	76	6	6	13	0.9	92.1	63.7	11	3.85	1.146	0.507	0.270
	53	6	6	12	1.8	85.0		6.7		0.876		
	82	7	7	111	.5	77.5		4.4		0.612		
	70	7	7	94	47	50.0		2.0		0.301		
	85	6	7	78	36	85.1		2.2		0.342		
	90	7	7	62	15	71.7		3.5		0.514		
	56	6	6	41	13	68.3		3.2		0.605		
	53	6	6	40	29	50.0		1.1		0.148		
	74	6	6	6	10	91.8		.6		0.415		
	123	7	7	10	4.6	76.2		4.2		0.673		
	81	7	7	18	6.2	65.6		2.0		0.46*		
	80	7	7	18	1.0	80.4		0.6		0.078		
	57	6	6	10	16	0		1.0		0.000		
33	86	7	7	14	2.4	82.9	75.0	6.8	0.63	0.763	1.777	0.170
	81	7	7	11	1.5	86.4		7.3		0.803		
	78	6	6	101	137	35.6		0.74		1.889		
	54	4	6	61	80	21.1		0.70		1.881		
	75	6	6	93	60	101		0.38		1.580		

TABLE II  
 FIVE DOSES OF MEDIUM ETHANOLIC EXTRACT UPON LEISHMANIA (STRAIN "11")  
 IN 1 FEMALE GUINEA PIG

Dose (mg Stry- chnine)	Weight (g)	Spleen Size		Parasit. Count per 100 nuclei		Mean Reduc- tion	Ratio Before After	Mean Ratio Before After	Log Ratio Before After	Mean Log Ratio Before After ( $\bar{y}$ )	Standard Devia- tion ( $\sigma_y$ )
		Before Treat- ment	After Treat- ment	Before Treat- ment	After Treat- ment						
800	60	6	6	180	0.20	98.0	92.0		0.74		
	65	6	6	100	1.6	97.0	100		2.000		
	61	4	6	125	0.10	99.0	120		3.114		
	60	5	4	140	0.10	99.9	140	79.1	3.078	-0.11	0.503
	100	7	7	110	0.05	99.9	110		3.312		
400	85	7	4	71	0.20	97.1	30.0		0.05		
	60	5	5	67	0.05	97.1	110		0.11		
	55	3	6	57	0.54	97.1	110		3.041		
	75	5	5	210	4	98.0	97		1.040		
	50	4	4	180	1.5	97.1	100		2.01		
200	55	5	5	130	0.75	97.4	140		2.55		
	60	4	4	120	0.40	98.4	98.9	79.9	2.778	13	0.431
	110	6	7	118	0.67	97.1	170		2.9		
	60	4	4	110	0.10	98.0	98.0		3.943		
	55	4	4	47	0.40	99.2	170		2.070		
100	90	6	6	110	0.57	97.3	37		1.188		
	75	4	6	40	18	9.5	13		1.116		
	99	7	7	110	84	19.1	1.2		0.078		
	74	6	6	65	0.51	98.2	140		2.078		
	60	4	6	65	13	98.0	19		0.090		
50	110	6	6	11	1.0	97.7	3.7		0.508	1.101	0.411
	60	4	6	13	0.71	94	13		1.433		
	60	6	6	40	0.71	95.0	56		1.748		
	80	5	4	42	1.2	95.0	40		1.201		
	125	7	6	11	0.31	96.9	32		1.405		
10	55	4	6	148	1	81.0	8.3		0.74		
	80	7	7	97	1.7	99.2	55		1.710		
	105	7	7	97	28.0	92.1	0.31		1.491		
	60	6	5	81	15	41.7	5.6		0.718		
	61	4	5	60	35	41.7	1.7		0.250	0.450	0.653
1	40	4	5	40	12	70.0	3.3		0.810		
	63	4	4	40	13	67.5	3.1		0.491		
	87	6	6	30	10	68.7	2.0		0.177		
	61	4	6	47	1.0	96.3	37		1.431		
	80	6	6	215	420	7.0	10.0		1.048		
0.1	65	6	6	170	140	18.5	3.1		1.873	0.7	0.7
	65	6	6	140	140	18.5	3.1		1.873		
	65	6	6	140	140	18.5	3.1		1.873		
	65	6	6	140	140	18.5	3.1		1.873		
	65	6	6	140	140	18.5	3.1		1.873		

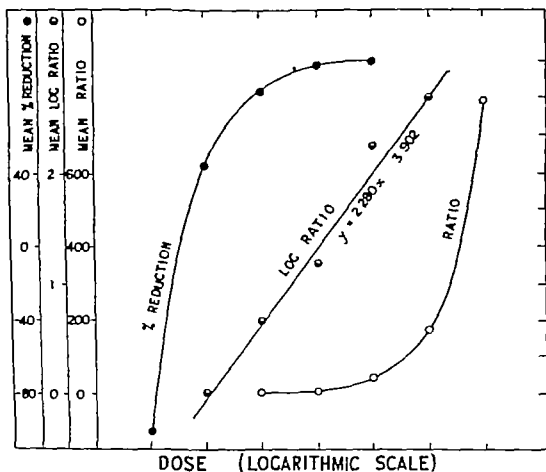


FIG. 2.—Dose-response curves for sodium stibogluconate (data from Table II). The curves are separated horizontally to avoid confusion. The logarithm of the ratio of parasite counts before and after treatment bears a linear relationship to the logarithm of the dose.

Fig. 2 shows the results of plotting the means of functions of the responses recorded in Table II against the logarithms of the corresponding doses. It will be seen that the percentage reduction in count

$$\left( \frac{\text{count before treatment} - \text{count after treatment}}{\text{count before treatment}} \times 100 \right)$$

is a fairly good measure of response in the lower dose range, but with higher doses the relationship is not linear. The reduction ratio

$$\left( \frac{\text{count before treatment}}{\text{count after treatment}} \right)$$

is linear only in the higher dose range. The best linear relationship for all dose levels is given by plotting the logarithm of the 'reduction ratio' against the logarithm of the dose. The regression line in Fig. 2 was fitted by the method of least squares. Other leishmanicidal drugs also give straight line graphs by this method.



## 2. Correlation between response weight degree of infection and spleen size

The correlation diagrams in Fig 3 have also been prepared from the data in Table II. The animals in each group are arranged in descending order of initial degree of infection, so that positive correlation between this and the other characters plotted would show as a downward trend to the right.

It will be seen that there is no correlation whatever between the degree of infection before treatment and the reduction ratio, weight, or spleen size. There is significant positive correlation between weight and spleen size

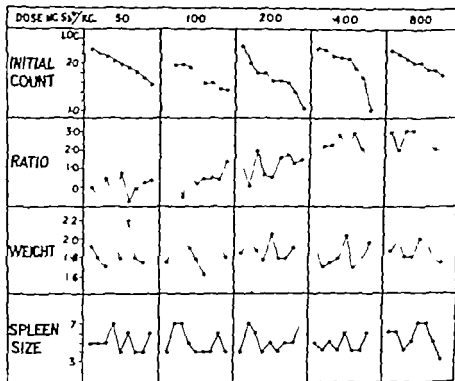


FIG. 3.—Correlation diagrams (data from Table II)

( $r = 0.698 \pm 0.079$  for the whole forty three animals). There is a suggestion of negative correlation between reduction ratio and weight, that is, that heavy animals may be more resistant to treatment than lighter ones. With the number of animals used in this test, the correlation is not significant (50 mg/kg dose,  $r = -0.43 \pm 0.29$  100 mg/kg dose  $r = +0.06 \pm 0.35$  200 mg/kg dose,  $r = -0.22 \pm 0.34$  400 mg/kg dose,  $r = -0.20 \pm 0.33$  800 mg/kg dose,  $r = +0.23 \pm 0.36$ ). Nevertheless, the conclusion can be drawn that it is important to use animals of as nearly equal weight as possible, and that when animals of varying weight have to be used it is probably more important to

distribute weights evenly among the groups than it is to group the animals according to the initial degree of infection. Further information upon this point is being collected.

The positive correlation between weight and spleen size is to be expected, but the results also show clearly that the size of the spleen is not proportional to the number of parasites it contains and that the spleen size is therefore useless as an indication of degree of infection. The size of the spleen was never reduced by single doses of drugs in the course of 1 week, but animals receiving large doses, and which were opened several weeks later sometimes showed a considerable reduction in size and lightening of colour of the spleen.

It is to be expected that if a proper selection of animals could be made from a large stock distributing litter mates among the groups and taking into account sex weight and degree of infection the variance due to individual differences in the groups would be considerably reduced. In spite of the mixed nature of stock used in the experiments recorded in Tables I and II the calculated values of  $t$  between the mean responses at different dose levels (Table III) show that doubling the dose produces an effect that is significant,

TABLE III

THE SIGNIFICANCE BETWEEN THE DIFFERENCES IN RESPONSE PRODUCED BY GRADED DOSES OF SODIUM STIBOGLUCONATE IN HAMSTERS INFECTED WITH *LEISHMANIA*  
(DATA FROM TABLES I AND II).

Strain	Dose (mg.Sbv/kg)	Mean Log Reduction Ratio (M)	Standard Error of M. (e)	$t = \frac{M_1 - M_2}{\sqrt{e_1^2 + e_2^2}}$
A	122	1.061	0.078	5.1
	66	0.507	0.077	
H	800	2.644	0.196	$\left. \begin{array}{l} 1.6 \\ 5.0 \\ 4.0 \\ 6.0 \\ 4.3 \\ 2.3 \end{array} \right\}$
	400	2.245	0.152	
	200	1.191	0.214	
	100	0.650	0.218	
	50	0.007	0.171	

\* A value of "t" of 2.0 and over is considered significant.

or on the borderline of significance and quadrupling the dose produces a highly significant result. This is an advance upon previous semi-quantitative methods of estimating leishmanicidal activity.

### 3 *The effect of sex*

Groups of animals of the same sex were used whenever possible but no great differences of response have been observed between male and female hamsters of the same age. On one occasion, some infected females were used for breeding during the incubation period of strain "A." The animals which had had litters were then significantly more resistant to drug treatment than males of the same age inoculated at the same time, and of approximately the same degree of infection. It is essential, therefore, if mixed animals have to be used to separate the sexes during the incubation period.

### 4 *Variation in the position of the dose-response curve from test to test*

There is considerable variation in the position of the dose-response curve in different tests, even with animals infected with the same strain of leishmania (Fig. 4). Therefore, when drugs are to be compared animals from the same infected stock must always be treated with a standard preparation. Sodium stibogluconate has been chosen as the standard in this laboratory as it is obtainable in stable solution at a convenient concentration.

### 5 *The effect of time upon the response to a single dose of drug*

The choice of the interval of 1 week for a drug to exert its effect was made as a result of a large number of experiments in which spleen fragments were removed repeatedly from animals after drug treatment. A few of the results are shown in Fig. 5. It will be seen that with strains "A" and "L" the effect of a dose was not always complete at the end of the 1st week, and a further reduction of count occurred during the 2nd, and sometimes the 3rd or 4th weeks. By the 6th or 7th week, the infection was again increasing except in animals which had received large doses—some of the latter became quite free from infection as tested by microscopical examination and cultures in Locke blood-agar and NNN medium. Some animals infected with strain "A" and most of those infected with strains "L" and "H" showed a maximum effect for small and moderate doses at the end of the 1st week and, if left for longer periods before assessment, the effects of doses would have been missed. Doses of antimonials which were insufficient to cause reduction of count in the 1st week seldom produced a significant effect when the animals were left for longer periods. Thus, for the assessment of the effect of small doses, and for convenience in routine tests, 1 week appears to be the best time to take. Longer periods may give greater responses to large doses, but the test becomes less convenient and the variation between the responses of individual animals is greater.

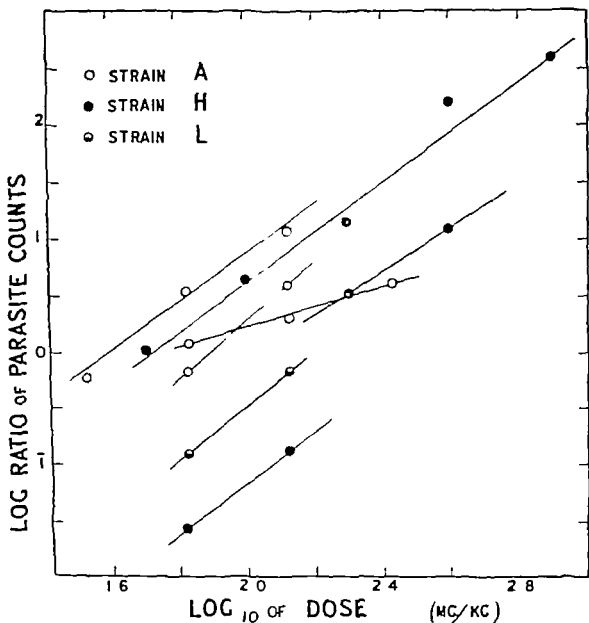


FIG. 4.—Dose response curves for sodium stibogluconate. The position of the line varies from test to test.

It must be emphasized that this argument should be applied only to experiments with antimony compounds. There are indications that single doses of aromatic diamidines exert their action for much longer periods than a week even in doses which allow the infection to increase during the 1st week, a significant reduction of count has been observed by the end of the 2nd or 3rd week.

#### DISCUSSION

It is puzzling that, with one exception the action of single doses of drugs upon experimental leishmaniasis has not been reported previously. When

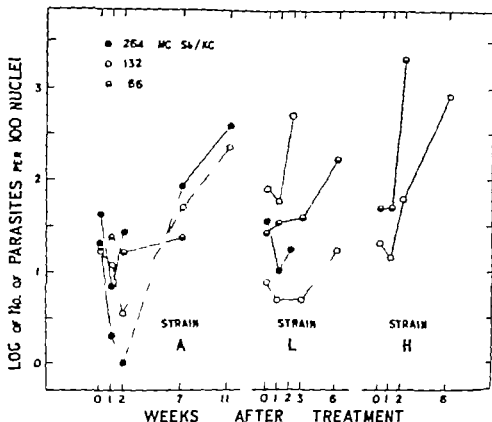


FIG. 9.—The effect of time of action of a single injection of sodium stibogluconate upon the degree of infection in the spleens of hamsters inoculated with different strains of leishmania. Each set of points connected by lines represents a set of observations on a single animal.

this effect was first observed with strain A, it was thought that this strain might have become susceptible to drug action by attenuation through long passage in culture. However the same behaviour was shown by the two freshly isolated strains, "L" and "H" one of which produced unusually rapid and heavy infection in the hamster. Attenuation of strain cannot therefore be the cause. The species of hamster used (*Cricetus auratus*) is the same as that used by Professor ADLER in Palestine, and by Dr FULTON in Liverpool and yet neither of these workers has reported activity with single doses of drugs. The exception mentioned above is a recent report by ESKUTH and SCHMIDT (1943) in which a single dose of an oily suspension of solusibom is said to have an effect upon infections in the European hamster (*C. ferretarius*) equal to that of eight injections of the watery solution. This action is attributed to the slower excretion of the oily suspension.

The results of the present investigation show that it is possible to obtain a quantitative dose response relationship for the leishmanicidal activity of single doses of drugs in the Syrian hamster. For simplicity all the experiments reported here have been made with sodium stibogluconate, but the results obtained with other quinquivalent antimonials (neostam, neostibosan, Ureastibamine) are qualitatively similar and one of these drugs could equally well have been used as an illustration.

A test of this kind does not, of course, provide a complete investigation of the leishmanicidal activity of a substance. It is rather to be regarded as a counterpart of the widely used trypanocidal test, in which the transitory effect of a drug upon the blood infection in the mouse is used to standardize therapeutic potency. This kind of test does not show whether or not a drug is capable of complete sterilization of an infection nor does it show the effects of repeated administration of small doses, though the technique can be modified to give information on these points. The main advantage of the method is that it is rapid, and gives a result for which the errors may be calculated.

SOONG and ANDERSON (1941) have suggested that thirty animals should be used on each dose for the assessment of leishmanicidal activity. The results of the present work indicate that much information may be obtained from the use of fewer animals if the data are analysed.

The next step is to compare the dose-response curves for various leishmanicidal drugs and to correlate the results as far as possible with clinical experience. The results of such experiments will be given in Part III of this work.

### SUMMARY

1. A dose response curve for the leishmanicidal activity of single doses of sodium stibogluconate has been obtained. A linear relationship has been shown to exist between the logarithm of the dose and the logarithm of the ratio of the parasite counts in spleen smears before and after treatment.

2. The effects upon the results of initial degree of infection, body weight, sex, and the size of the spleen have been considered.

3. The effect of allowing the drug to act for varying lengths of time before assessing the results has been investigated. A period of 1 week appears to be most suitable for the testing of antimony compounds.

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## AN INVESTIGATION ON NEW REPELLENTS FOR THE PROTECTION OF MAN AGAINST MOSQUITO ATTACKS

BY

D. BLAGOVESCHENSKY

N. BREGETOVA

AND

A. MONCHADSKY

*From Moscow*

The repellent nets devised by Academician EUGENE PAVLOVSKY proved to be extremely reliable in the protection of human beings from the attacks of mosquitoes and other blood sucking insects. Various repellent pastes, ointments and emulsions, which are applied to the skin, are effective only for a short period of time and their deterrent action is inadequate. However an increase in the content of the active principle is impracticable since it causes irritation of the skin, especially if this is moist from perspiration. As to mosquito-veils, though these afford mechanical protection, they are too unwieldy and therefore hamper the movements and are not very portable moreover they diminish the visual and auditory powers. The repellent nets are free from these defects and can be successfully employed against mosquitoes, midges and sandflies. Under war conditions their importance for the Red Army troops, river flotillas and frontier guards is especially great.

During the war it became necessary to find some new repellents, which could be produced from local raw materials and used for impregnating the nets. Our investigations, which were carried out in Middle Asia, had this object in view. From a number of preparations (essential oils and by products of their purification) obtained from the "Efironas" Works at Pakhtabad we selected—after preliminary tests—the d- $\alpha$ -pinene fraction of juniper oil. As regards the other preparations, the results obtained with some of them were negative, while others were produced in too small quantities.

Juniper oil is obtained from the young branches of *Juniperus seravschanica* Horn. which is widely distributed in Middle Asia at altitudes above 2,000 metres. Its constituents include pinene, myrcene and cedrol. Cedrol represents a very valuable medicinal preparation whereas d- $\alpha$ -pinene which is recovered in considerable amounts as a waste product in the course of its purification, has not hitherto been utilized, on account of which the cost of production remained high.

We have tested pinene both in its pure form and as a substitute for turpentine in PAVLOVSKY's formulas for repellent mixtures. After a number of preliminary experiments our choice fell on the following two preparations in which turpentine was substituted by



d- $\alpha$ -pinene (1) " naphtha-lysol 20 parts, pinene 10 water 70 and (2) lysol 15 parts, pinene 8 water 77. Our method of preparation of the mixture and of impregnation of the nets was the same as that recommended by E. PAVLOVSKY.

### TESTS OF REPELLENTS FOR THE INDIVIDUAL PROTECTION OF MAN UNDER NATURAL CONDITIONS.

The tests were carried out in the flood plain of the left bank in the lower reach of the river Vakhsh (Dzhulikul region Stalinabad Province, Tadzhik S.S.R.) in a jungle zone thickly overgrown with oleasters, poplars and beech. In the course of these tests parallel control counts were made of mosquitoes attacking human beings under natural conditions since an exact estimate of the effectiveness of the nets soaked in the test substances could only be arrived at by comparing under identical conditions, the number of mosquitoes attacking, on the one hand, persons protected by the net, on the other those not protected.

The control counts of mosquito attacks were made throughout the entire period of 24 hours by means of a recording apparatus which ensures absolutely complete and objective results with a simultaneous record of the fundamental meteorological factors. The tests of the repellents, together with the parallel control counts, were conducted during the period when mosquitoes were attacking most actively. The tests were made by one or two men, whose heads were covered by the impregnated net in the form of a hood leaving the face exposed. The observers, who were placed near the control points, recorded the number of mosquito bites in the region of the face and neck, which were the parts it was intended to protect by the net. Each experiment lasted 15 to 30 minutes.

According to the data obtained from 403 control counts, the number and specific composition of the attacking mosquitoes were as follows: during a count lasting 5 minutes the average number of mosquitoes attacking one man was eight, while the maximum was 127. The predominant mosquito was the very active blood-sucker *Mansonia richiardi* (91 per cent.) while the minority belonged to *Anopheles superpictus* Gr. and *A. hyrcanus* Pall. (4 per cent.) as well as to species of the genera *Aedes*, *Culex* and *Theobaldia* (5 per cent.). The insignificant proportion of malaria vectors among these mosquitoes does not indicate their absence, for in human dwellings they constituted 70 per cent. but it points to their anrophilism and adaptation to attacks in dwellings.

The total number of experiments with nets soaked in pure d- $\alpha$  pinene fraction and in a mixture of this with lysol or naphtha lysol was 59. (See Table I.)

A comparison of the number of mosquitoes in the direct experiments with those in the controls reveals the high effectiveness of the repellents tested. The effect of the nets lasts 5 to 6 days. Attempts to prolong the effective period by addition of ten parts of a vegetable oil to the mixture have so far remained inconclusive.

TABLE I.

Test Mixture.	Total Number of Experiments.		Total Duration of Experiments.		Number of Attacking Mosquitoes in Experiments.	
	Direct.	Control.	Direct.	Control.	Direct.	Control.
d- $\alpha$ -pinene	14	14	3h. 40m.	1h. 10m.	3	156
"Naphtha-lysol"						
pinene	38	38	15h. 20m.	3h. 10m.	8	512
Lysol-pinene	"	7	1h. 50m.	35m.	1	112

## TESTS OF REPELLENTS FOR THE PROTECTION OF BUILDINGS

These experiments were carried out at a collective farm of the Stalinabad district in an area infested with *Anopheles superpictus* which is the most dangerous malaria vector in Tadzhikistan. At that time the cold autumn weather caused an increase in the numbers of mosquitoes entering houses, thereby bringing about an autumnal rise of the curve of fresh infections. In Table II are shown the results of catches with the help of an aspirator each lasting 10 minutes. They were made in some of the buildings of the collective farm, and illustrate the degree to which the houses are populated with mosquitoes.

TABLE II

Building	Date of Observation	Time of Observation	Numbers of <i>A. superpictus</i>		
			Females.	Males	Total.
Dwelling	1st October	18.30 to 18.40	45	49	75
Empty cow-houses	4th "	12.20 to 12.30	66	98	164
	6th "	15.20 to 15.40	84	83	167
	9th "	12.25 to 1* 45	66	90	185
Stables	5th "	15.20 to 15.30	76	29	105
	9th "	16.25 to 16.45	67	10	77

In counts made in the open country with the help of the recording apparatus, the only species reported was again *A. superpictus* but it occurred in very small numbers, only ninety-two mosquitoes having been collected in the course of 172 counts. In this case the predilection of this species for dwellings and its tendency to attack chiefly in these, and not in the open are clearly marked.

Tests were also made in a hut (*kibitka*) of the local type, with clay walls an earthen floor and rush thatched roof. Daily catches were made of all the

mosquitoes present in the house between 18.30 and 19.30. During the period the windows were screened with muslin, while at other times they were left open. On the days when the tests were conducted the windows were screened with netting having meshes of  $1.5 \times 1.5$  cm. and soaked in the solution to be tested. The door remained closed and the entrance was curtained with muslin. (See Table III.)

TABLE III

Date.	Time.	Number of Mosquitoes	Nature of Observation.
1st October	18.30 to 19.30	78	Control count.
2nd		19	Control count.
2nd	19.30	Nil	Window protected by net soaked in $\alpha$ -pinene.
3rd	18.30 to 19.30	4	Experiment.
4th	"	3	Experiment.
5th		18	Control count.
6th		7	Control count.
6th	19.30	Nil	Window protected by net soaked in a mixture of naphtha-lysol and $\alpha$ -pinene (No. 2).
7th	18.30 to 19.30	1	Experiment.
8th	"	1	Experiment.
9th		19	Control count.

The results can be regarded as highly satisfactory since even dwellings in which the windows are screened by wire gauze netting and the doors protected by a porch are not fully ensured against the entry of single mosquitoes.

It has thus been demonstrated that the  $\delta$ - $\alpha$ -pinene fraction of juniper oil is a very effective repellent substance. It can be employed both in its pure form or as a substitute for turpentine in the formulas of Academician E. PAVLOVSKY. The practical application of this substance is recommended also because there is a fairly abundant source of raw material and ample possibilities for its production.

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# THE IDENTITY OF THE CILIATE *BALANTIDIUM MINUTUM* AN ALLEGED PARASITE OF MAN

BY

J. M. WATSON A.R.C.S.,\*

Lecturer in Zoology Northern Polytechnic

(From the Wellcome Laboratories of Tropical Medicine London)

Perhaps the principal interest which coprozoic protozoa hold for the medical man is the possibility which exists of incautiously mistaking them for parasitic forms. Many classical instances may be found in the literature on the intestinal protozoa in which forms now known to be free-living coprozoic species have been described as human parasites. A critical discussion of these forms may be found in the works of DOBELL and O'CONNOR (1921), WENYON (1926) and elsewhere. HOARE (1927) made a detailed investigation of the case of *Uronema caudatum*, which had been alleged by MARTINI (1910) and by YAKIMOFF and his collaborators (1921-1922) to be both parasitic and pathogenic, and he was able to show that the forms described by these workers actually belonged to at least two different species of well known, free-living ciliates obviously coprozoic forms present in the faeces as contaminants.

Of all the doubtful cases of human parasitism, perhaps the best known and at the same time the most mysterious and controversial is that of *Balantidium minutum* Schaudinn. This ciliate was originally described by SCHAUDINN in 1899 his specimens being obtained in a sample of human stool from a patient suffering among other things, from diarrhoea. It has only been reported a few times since, a remarkable fact in view of the immense number of stool examinations made annually in connection with dysentery diarrhoea and helminthic infections and for many years has been regarded as of

\* I am deeply indebted to the authorities of the Wellcome Foundation, to the Director-in-Chief of the Wellcome Research Institution and to the Director of the Wellcome Laboratories of Tropical Medicine for extending to me the use of their admirable facilities in the execution of this work. I am also profoundly grateful to Dr. C. A. HOARE for his unfailing help and encouragement.

doubtful status. SANGIORGI and UGDULENA (1917), SANGIORGI (1919), PISTO (1919), and more recently MATTHEWSSIAN (1928) have recorded it from human faeces, but I have been unable to trace any further mention of its occurrence. As early as 1922, BRUMPT suggested that this ciliate was really a coprozoan organism, and a careful study of the figures and descriptions given by SCHAUDIN and by SANGIORGI has convinced me, not only that this is the case but also that the organism recorded and described by these observers was actually in all probability the free-living ciliate *Balantiothrus muris* Schewiakoff. This conclusion is supported by the fact that WIGHT (1925) records that a ciliate, subsequently identified by Dr C. DOBELL, F.R.S., as *Balantiothrus muris* had been reported, both from human faeces and from the water supply in the San Francisco district of California, as *Balantidium muris*.

In order to implement this conclusion a careful comparison of the structural details of the two species is given below followed by a critical discussion of the apparent discrepancies and of the other reports of occurrence.

Since a summary of SCHAUDIN's description and a copy of his figure appear in such standard works as those of DOBELL and O'CONNOR (1921), CRAIG (1926), WENTON (1926), KNOWLES (1928), THOMSON and ROBERTSON (1929) and KUDO (1939), and since the original paper itself (JAKOBY and SCHAUDIN 1899) is readily available, it is unnecessary to give a detailed account of *Balantidium muris*. It must, however be pointed out that the copies of SCHAUDIN's figure given in some of the above works do not show the peristomal membrane which appears distinctly though faintly in the original.

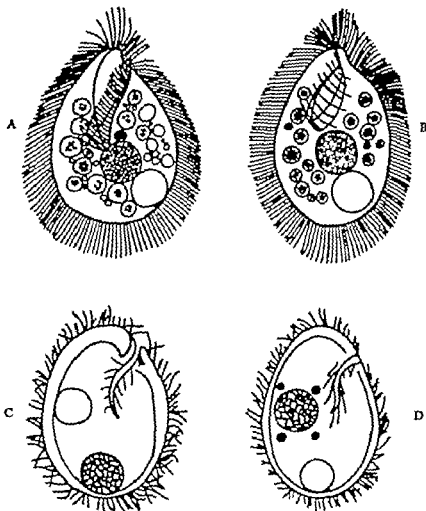
The comparative table on p. 153 is based upon SCHAUDIN's original description of *Balantidium muris* and upon my own description of *Balantiothrus muris* (WATSON 1940).

A perusal of the table and reference to Figures A and B show the *Balantidium muris* resembles *Balantiothrus muris* within the limits of observational error in general form and dimensions, in the position of the peristome, in the position and transverse motion of the adoral cilia, in the length and general appearance of the body cilia, in the appearance and contents of the food vacuoles, in the position of the cytoproct, in the position of the contractile vacuole and the macronucleus, and in the form of the micronucleus. The two organisms appear to differ on the other hand, in the structure and size of the peristome, in the position of the discharge-pore of the contractile vacuole, in the shape of the macronucleus, in the position of the micronucleus and in the form of the cysts.

There is evidently then, a very close resemblance between the two ciliates, marred only by a few apparent differences which, however upon closer examination prove to be of but slight and doubtful significance. Thus in the first place, with regard to the peristomal apparatus, the membrane figured by SCHAUDIN closely resembles the partially retracted hood of *Balantiothrus*. The anterior extension of the peristomal depression to the apex of

TABLE.

	<i>Balantidium manitum</i> .	<i>Balantiophorus minutus</i>
Shape	Oval in young forms, pyriform in mature individuals. Anterior pointed end slightly bent to left or right, opposite side sloping obliquely in opposite direction. Shows considerable power of change of shape.	Usually ovoid, rounded at both ends, anterior end being narrower more markedly so in mature individuals. Anterior extremity bent ventralwards, dorsal side sloping obliquely in opposite direction. Shows considerable power of change of shape.
Dimensions	20-32 $\mu$ long $\times$ 14-20 $\mu$ wide. Generally about 3:2.	Very variable 12-44 $\mu$ long $\times$ 7-33 $\mu$ wide—mean about 32 $\times$ 21 $\mu$ . Generally about 3:2.
Peristome	Narrow ventral slit, wider in front, pointed posteriorly. Shallow in front, deeper behind. Occupies whole width of oblique inclination of anterior margin and extends back as far as equator of body being slightly diverted to the right posteriorly. Right margin sharply defined bearing ordinary cilia. Left margin less distinct, produced into thin transparent, triangular membrane narrower in front, which can be folded over the peritomal cavity. Adoral cilia on left margin which beat inwards. No cilia in peristome. Performs opening and shutting movements (like a mouth) in living animal.	Spoon-shaped depression with narrow end anterior. Situated in middle of anterior half of ventral body surface. Shallow in front, deeper behind. Thin, transparent sac-like membrane narrow in front, wider behind attached to left, posterior and right margins with anterior opening. Membrane conspicuous when expanded but can be retracted into peritomal cavity being then invisible. Adoral cilia on left margin which beat inwards. No cilia in peristome. Right margin bears ordinary body-cilia.
Cilia	Thin, long (7-8 $\mu$ ), forming a dense covering. No distinct striation of body. Apparent anterior tuft of longer cilia bent ventralwards.	Thin, long (5-8 $\mu$ ) and setiform forming a dense covering. No distinct striation of body. Apparent anterior tuft of longer cilia bent ventralwards in some individuals.
Food vacuoles	Small, contain fine granules.	Small, contain minute bacteria.
Cytoproct	Not distinct, but indigestible particles expelled posteriorly.	Posterodorsal—clearly shown only in silver preparations.
Contractile vacuole	Single posterodorsal and slightly to left in position with posterodorsal discharge pore.	Single posterior, with ventral discharge pore.
Macronucleus	Spherical central in position with distinct membrane and granular chromatin. 6-7 $\mu$ in diameter.	Generally elliptical central in position with distinct membrane and granular chromatin. 8 $\mu$ in length in an average specimen.
Micronucleus	Single spherical lying close against macronuclear membrane on anterior side. 1 $\mu$ in diameter.	Single spherical generally in small depression of upper surface of macronucleus but position varies. 1-2 $\mu$ diameter.
Cyst	Mature cyst generally oval.	Mature cyst irregularly spherical with rugose surface.



- A. *Balantidium minutum* (after SCHAUDINN).  
 B. *Balantophorus minutus* (original camera lucida drawing from an unstained, formalin-fixed specimen).  
 C. *Balantidium minutum* sp. *albicans* (after SANGHVI).  
 D. *Balantidium minutum* sp. *analeum* (after SANGHVI).

the body as described by SCHAUDINN is an error which could easily be made in relation to *Balantophorus* since the pre-oral keel of the latter and the fact that anteriorly the margin of the peristomal depression is not sharp but merges gradually with the body surface (already sloping in the same direction) make it easy to imagine a narrow extension of the peristome to the tip of the body. More especially is this the case when SCHEWLAKOFF's sodium carbonate

technique is employed as it was by SCHAUDINN. Further SCHAUDINN describes the peristome as opening and shutting like a mouth which implies a somewhat variable shape, I have never observed such movements in *Balantio-phorus* but they might have been simulated by the raising and lowering of the peristomial membrane. In this connection it may finally be remarked that it is exceedingly difficult to get an accurate notion of the structure of the peristome in *Balantio-phorus minutus* the membrane being practically invisible in living and unstained specimens and generally much distorted in sodium carbonate preparations the only really satisfactory technique in my experience being the silver method of Klein by which both the root and margin of the membrane were well shown. In the second place, the position of the discharge pore of the contractile vacuole is difficult to determine on account of the small size of the structures. I was only finally convinced of its position in *Balantio-phorus minutus* by observing some China blue preparations in which the stain had completely filled the vacuole and discharge-pore evidently caught in the act of discharge. In the third place, with regard to the nuclei the form of the macronucleus in *Balantio-phorus minutus* is variable, and I should not be surprised to find that in a particular strain it was generally spherical rather than ellipsoidal while the position of the micronucleus is also liable to change, and is certainly not a characteristic on which any specific distinction should be based. Lastly there remains only the shape of the cyst. In this connection it is noteworthy that SCHAUDINN studied a mixed infection of *Nyctotherus faba* and *Balantidium minutum* and makes no mention of having made any cultures of either species alone. He says that the cysts of *N faba* were also oval and indistinguishable from those of *Balantidium minutum* except by the structure of the nucleus which contained large chromatin masses in *N faba* smaller granules in *B minutum*. Moreover no description or figure of the cysts of either species is given, apart from the bald statement that they are oval it is not even stated whether the cyst-wall was thin or thick, or whether it had a smooth or wrinkled surface. It seems to me likely that, in fact, SCHAUDINN never saw any cysts but those of *N faba*. *Balantio-phorus minutus* rarely forms cysts in the laboratory under ordinary conditions.

A further relevant point is the fact that the dimensions and proportions of *Balantidium minutum* as described by SCHAUDINN in the text, do not conform to those shown in the figure. If the latter be redrawn to conform with the text it bears a particularly close resemblance to the bursaria-form of *Balantio-phorus minutus*.

With regard to the circumstances under which *Balantidium minutum* was found, it should be noted that the patient from whom the stools were obtained was a German waiter 30 years of age who had recently crossed the Atlantic and resided for four years in St. Louis and New York. He was admitted to hospital suffering from abdominal pains accompanied by alternating attacks of diarrhoea and constipation with pain during defaecation and intense anal pruritus. He had previously suffered from malaria and gonorrhoea, and while



in the hospital showed inflammation and haemorrhage of the lungs. In addition to the ciliates the stools contained numerous eggs and larvae of *Ancylostoma*, which would probably account for the abdominal pain and anal pruritus. The patient was apparently cured of diarrhoea by administration of quinine for 3 days, after which he left hospital and disappeared.

JAKOBY states that the stool was taken directly from the intestine for the purpose of examination, but he does not state whether it was received into a sterile vessel whether it was diluted with tapwater or any other medium nor whether it was examined immediately or left to stand for some time. The occurrence of a second infection with *Balantidium minutum* from another patient in the same clinic seems to indicate that the source of the ciliates in the stools was perhaps the tapwater used to wash the bedpans or to dilute the stools. WRIGHT (1926) found *Balantophorus* to occur in tapwater at California and says it was often reported from faeces as *Balantidium minutum*. If this mistake could occur with modern methods and in the light of more up-to-date knowledge, how much more likely is it that it could have occurred 30 years earlier.

The only remaining difficulty in identifying *Balantidium minutum* with *Balantophorus minutus* now seems to be the fact that the former was, according to JAKOBY and SCHAUDIN, only found in liquid diarrhoeic stools and disappeared from the normal formed stools. My experiments upon *Balantophorus minutus*, however, have shown that it is not able to survive and multiply for long in liquid stools (WATSON 1945). This circumstance probably accounts for the rarity with which *Balantidium minutum* has been subsequently reported, since it is generally these abnormal stools which are examined. Two possible explanations of the apparent discrepancy in the original case present themselves. On the one hand the stools of JAKOBY's patient may although liquid have had a low osmotic pressure and been devoid of bile in which case *Balantophorus minutus* might have been expected to survive in them. On the other hand, even if the stool was not diluted—thereby enabling *Balantophorus minutus* to survive in it for some time and even to multiply—it may have been examined fairly soon after passage, in which case, as my experiments have shown (WATSON 1945) the ciliates would still be alive.

In the latter part of his paper SCHAUDIN remarks that a further stool sample from another patient at the same clinic was brought to him by Dr. SCHULZ, and that this sample also contained ciliates which proved to be *Balantidium minutum* again. SCHAUDIN says that as far as he knows SCHULZ himself would give a report of this second case. SCHULZ's account appeared shortly afterwards (1899) but in it he states that he at first thought his ciliate was *Balantidium protozoon* [sic] but that upon consulting Dr. SCHAUDIN he was informed that it was *Colpoda cucullus*. This extraordinary discrepancy has already been remarked upon by DOBELL and O'CONNOR (1921) who very reasonably infer that it raises doubts with regard to the identity not only of

the ciliate in question but also of the ciliates originally described as *Balantidium minutum*. SCHULZ's paper is hardly convincing. No figure is given and the extremely meagre description could apply not only to *Colpoda cucullus* to *Balantidium minutum* to *Balantophorus minutus* but also to any one of a considerable number of free-living ciliates. Hence, after this lapse of time, it is impossible even to make a conjecture as to the identity of the organism with which SCHULZ was dealing. The patient in question was suffering from a high grade anaemia, which SCHULZ believed to be due to the ciliates and it is suggested that the infection was contracted by drinking pond water containing much decaying organic matter which apparently induced vomiting abdominal pain, headache and progressive weakness. The diarrhoeic stools which were examined also contained *Giardia intestinalis*. According to HARTMAN and KYSER (1941) in a small percentage of *Giardia* infected individuals, symptoms similar to those described by SCHULZ may occur. Or more probably the condition of the patient was due to some bacterial infection which was not traced. In any case it is quite certain that it was not due to free living ciliates ingested with the pond water as any such organisms would mostly be destroyed by the digestive juices of the alimentary canal and any survivors could not live and multiply for long at the temperature of the human body. Rounded and egg shaped cysts with a double wall are also mentioned as occurring in the faeces. It will be remembered that the cysts of *Balantophorus minutus* are irregularly rounded and have a double wall. No statement is made as to how the stool samples were collected, whether they were diluted or whether they were examined immediately or after an interval. SCHULZ attempted to cultivate the ciliates in broth, urine and faecal suspension but the results were unsatisfactory. Possibly although temperature is not mentioned these cultures were maintained at blood heat, in which case no free living ciliate would be likely to survive.

In view of such evidence as is available it must be concluded that SCHULZ's ciliate was a free living form which contaminated the faeces after their passage, that its identity cannot now be determined, and that the contradictory statements of SCHAUDINN and SCHULZ with regard to it throw serious doubt on the identity of the original *Balantidium minutum*.

Five other reported cases of the occurrence of *Balantidium minutum* remain to be considered. The first of these in chronological sequence is that of BROOKS (1903) who refers to the finding of *Balantidium minutum* by Dr RUSSELL in the stools of soldiers in Porto Rico but as no details or figures are given and no further account appears to be available this case may be dismissed on the grounds of insufficient evidence.

No further report of its occurrence appeared until 1917 when SANGIORI and UGDULENA published a brief account of a ciliate which they obtained from the diarrhoeic faeces of a soldier. This organism, which varied in size from  $28.8 \times 11.2\mu$  to  $36.8 \times 25.6\mu$  and formed oval (?) cysts measuring  $12.8$

$\times 112\mu$  is stated to have corresponded to *B. minutum* of JAKOBY and SCHAUDINN from which it differed only in the eccentric position of the macronucleus and in the peculiar orientation of the micronucleus. The authors proposed to call it "*Balantidium minutum* sp. *Italicum*" [sic] SANGIORGI and UGDULENA had no difficulty in cultivating this ciliate by sowing a loopful of fresh faeces in peptone water the cultures being allowed to remain at room-temperature, and subinoculating weekly. The organisms showed rapid development and became extremely numerous, encystment occurring after 10 days. Cultures were also obtained on the surface of ordinary agar. These facts indicate that the organism in question was actually a coprozoic but not an entozoic form and it had presumably gained access to the initial culture, or to the faeces, by contamination. The description of the ciliate would apply equally well to *Balantiphorus minutus* as to *Balantidium minutum*, while the dimensions given for the cysts are consistent with the possibility that they were irregularly spherical, as in *Balantiphorus minutus* rather than oval. Unfortunately no figures are given.

Two years later SANGIORGI (1919) reported the occurrence of a form closely resembling *Balantidium minutum* in samples of water taken from wells or pools at Valona. The ciliates multiplied enormously in the samples examined. Morphologically they were stated to differ from JAKOBY and SCHAUDINN's species in the greater thickness of the ectoplasmic layer and in the displacement of the contractile vacuole and nucleus. From the figure it appears as though the latter feature might be the outcome of misinterpretation. In this case it is evident that the author was dealing with a free living ciliate, and his figures, though crude might well represent *Balantiphorus minutus*. It is not without significance that the copy of JAKOBY and SCHAUDINN's figure of *Balantidium minutum* given by SANGIORGI is grossly inaccurate. SANGIORGI proposed to call this organism "*Balantidium minutum* sp. *Albanense*". The dimensions given are consistently similar to those of *Balantiphorus minutus*. Oval cysts,  $12.5 \times 10.2\mu$  are stated to have been formed.

PRATO (1919) recorded the finding of *Balantidium minutum* in five specimens of faeces out of a total of 3,917 examined at various places in the State of Parana, Brazil. No figure or description of the organism so named is given, so that this case like that of BROOKS (1903), must be dismissed for lack of sufficient evidence.

WIGHT writing in 1926 refers to the fact that reports of the findings of *Balantidium minutum* in human faeces have been made from time to time in California, but considers all these reports to be errors based upon contamination of the faeces with *Balantiphorus minutus* which is stated to occur in the water supply.

Subsequently as far as I have been able to discover only one report of the occurrence of *Balantidium minutum* has been made. MATHEWSON (1928), in an examination of stools of 692 individuals from twelve villages

in Armenia and Transcaucasia found *B. minutum* in a single case. Unfortunately, he gives no figure or description, merely remarking that he found *Balantidium minutum* in the soft faeces of a 23-year-old peasant, but was unable to question him and investigate the case further.

In conclusion, then, it may be stated that careful comparison of SCHAUDINN's figure and description of *Balantidium minutum* with *Balantiophorus minutus* shows that in all essential features the two organisms are almost identical. Hence it may be fairly assumed that JAKOBY and SCHAUDINN's faecal specimens had become contaminated after discharge from the body of the patient, with *Balantiophorus minutus* which being a coprophilic organism subsequently multiplied enormously in the faeces. There are, it is true, slight difficulties in correlating the description of the peristomial apparatus in *Balantidium minutum* with this feature in *Balantiophorus minutus*, which are possibly due to the inaccurate results sometimes obtained by the older techniques. In any case, as SCHAUDINN made some remarkable statements—which have since been disproved—about the coprozoic and intestinal amoebae of man it is permissible to suppose that he may also have been mistaken in this case. BRUMPT (1922) and WENYON (1926) have both suggested that SCHAUDINN's species was a coprozoic form. The most striking confirmation of this suggestion has come from WIGHT (1926) who recorded that a ciliate identified as *Balantiophorus minutus* occurred from time to time in the water supply of the San Francisco district of California, and becoming a contaminant in faecal specimens and cultures had been reported on a number of occasions as *Balantidium minutum*. THOMSON and THOMSON (1918) recorded the finding of a "species of *Balantidium* or *Nyctotherus*" in human faeces which they were also able to cultivate from the dry sand of Egypt: their figure more closely resembles *Balantiophorus minutus* than any known species of *Balantidium* or *Nyctotherus*. SANGIORGI (1918) describes the culture of *Balantidium minutum* and several other species, now believed to be coprozoic protozoa, *in vitro* at laboratory temperature. Examination of the other reports of occurrence of *Balantidium minutum* does not reveal a single instance in which the evidence is inconsistent with the organism in question being a coprozoic species and most probably *Balantiophorus minutus*.

#### SUMMARY

A careful comparison is made between the characteristics and structure of *Balantidium minutum* Schaudinn and the free-living coprozoic ciliate, *Balantiophorus minutus* Schew, as a result of which it is concluded that these two forms are identical and that SCHAUDINN was actually dealing with faecal samples contaminated by free-living coprophilic protozoa. All subsequent cases in which *Balantidium minutum* is alleged to have occurred are discussed and it is shown that in no one of them is the evidence inconsistent with the organism in question having actually been *Balantiophorus minutus*.

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# OBSERVATIONS ON THE COPROPHILIC HABITS OF A CILIATE *BALANTIOPHORUS MINUTUS* SCHEWIAKOFF

BY

J. M. WATSON, A.R.C.S. \*

*Lecturer in Zoology, Northern Polytechnic*

*(From the Wellcome Laboratories of Tropical Medicine, London)*

As is well known there is a well-defined ecological group of protozoan organisms which are capable of living and multiplying within discharged faeces—the so-called coprozoic protozoa. Some years ago (1934) a sample of human stool was sent to these Laboratories for the identification of certain protozoa which were present in it. These proved to be a flagellate (*Bodo*) and a ciliate (*Balantiophorus minutus*) both obviously coprozoic organisms. A specimen of the stool was cultivated on agar and after some time the flagellates disappeared, leaving the ciliate in pure culture in which it has been maintained ever since. This seemed to me a suitable opportunity to investigate the behaviour of a coprozoic organism in relation to environmental factors and especially with regard to its coprophilic habits. Investigations along these lines with special reference to the medical aspect are, as far as I have been able to discover, confined to HOARE's paper on species of *Lembus*, *Cyclidium* and *Uronema* (HOARE, 1927). Other work on the coprozoic protozoa is very limited and mainly academic in outlook.

\* I am deeply indebted to the authorities of the Wellcome Foundation, to the Director-in-Chief of the Wellcome Research Institution and to the Director of the Wellcome Laboratories of Tropical Medicine for extending to me the use of their admirable facilities in the execution of this work. I am also profoundly grateful to Dr C. A. HOARE for his unfailing help and encouragement.

One question of medical interest which it was desired to solve was the ability of the ciliate to live and multiply in undiluted faeces, especially in the abnormal or liquid stools characteristic of various pathological conditions, such as diarrhoea and dysentery. Accordingly arrangements were made to obtain samples of human faeces, from both healthy and diseased subjects, which were then inoculated with the ciliate.\*

These faecal samples represented a wide variety of copric conditions, there being available in all twenty nine specimens from healthy individuals and from cases of seven different types of disease (amoebic dysentery, giardiasis, Flexner's dysentery, Sonne dysentery, ulcerative colitis, pellagra and nutritional diarrhoea).

These stools were classed according to their consistency and appearance as normal (brown and formed), loose (brown and semi-solid), diarrhoeic (soft to liquid and brown, yellowish or greenish in colour), dysenteric (loose or liquid and containing blood and mucus) and soapy (soft and of a mustard like consistency containing no definite particles). Each specimen was divided into two portions, one of which was inoculated with the active ciliate whilst still in its original condition, the other was diluted with ten times its volume of water and the suspension so obtained was inoculated with the ciliate.

It was found that *Balantrophorus minutus* grew well in normal faeces both before and after dilution, achieving indeed, such a fantastic abundance as some cases as to indicate that it is exceptionally well adapted to this habitat. A term was eventually put to its growth by the gradual drying up of the stool. Since stools from cases of intestinal disorder are generally more fluid than normal stools, and since artificial dilution of the faeces is favourable to the growth of the ciliates, it might have been expected that even better growth would have been obtained in pathological stool samples. Such, however, was not the case. Not merely did *B. minutus* fail to grow in any undiluted abnormal stool whether loose diarrhoeic or dysenteric, but, with one exception, it proved to be impossible to recover it from such faeces by inoculating portions of them into so favourable a medium as dilute Lemco broth. This remarkable fact, which was also noted by HOARE (1927), now invited investigation.

The factors inhibiting growth of *B. minutus* in abnormal faeces proved to be numerous and it is by no means certain that those which I was able to determine as affecting the issue formed a complete list. One of the most obvious inhibiting factors was the presence of urine, indicated by the presence of crystals of ammonium magnesium phosphate in the faeces. Tests showed that urine was highly toxic to the ciliate, since as little as 2 per cent. when added to the culture medium, or when mixed with faeces which were subsequently inoculated with the ciliate, proved fatal. When a drop of urine is

\* I am indebted to Dr W. BROUGHTON ALCOCK, Pathologist of the West Middlesex Hospital, to Dr ARTHUR DAVIES, Pathologist of the Seamen's Hospital, Greenwich, and to Major T. CRAWFORD, of the Military Hospital at Shenley for their courtesy and co-operation in providing me with material.

added to a drop of culture medium containing the active organisms under the microscope, they are almost instantaneously killed, the dead body assuming a characteristic distorted, shrunken and wrinkled appearance. That urine should have so pronounced and startling an effect was surprising since it has been reported (MALIWA and HAUS 1920) that *Balantidium coli* occasionally occurs abundantly in the urinary tract, and since also TUNNICLIFF (1929) used the ciliate *Parametium* for studying toxins from the urine of scarlet fever, measles and diphtheria patients. More detailed investigation showed that the lethal influence of urine upon *Balantophorus minutus* was primarily due to its high osmotic pressure and to a lesser degree, to the toxic effect of ammonia present in the urine. It was also found that urea has a depressing effect on the reproduction rate of the ciliate.

Another inhibiting factor was found to be the presence of traces of drugs in the faeces. 1 per cent. solutions of emetine hydrochloride, stovarsol, quinoxyl and yatren produced death with greater or less rapidity and 0.1 per cent. solutions of these drugs were sufficient to inhibit growth.

Still another inhibiting factor was found to be an unusually high osmotic pressure. According to CAMBRIDGE (1914) diarrhoeic and dysenteric stools frequently have an osmotic pressure higher than normal. Experiments with Beckmann's apparatus for determining the depression of the freezing point soon showed that this was indeed the case, and further tests conclusively demonstrated that *B. minutus* is not able to withstand solutions of equivalent osmotic pressure but rapidly succumbs to plasmolysis.

A fourth inhibiting factor of considerable potency was found to be the presence of unaltered bile salts in diarrhoeic and dysenteric stools. As is well known in normal stools cholate of soda is the only representative of the bile salts, from which it is formed during the passage of the intestinal contents through the bowel. In cases of dysentery and diarrhoea however the abnormally fluid stools are often due to the too rapid transit of the intestinal contents through the gut, so that unaltered bile salts may occur in the faeces. That the least trace of bile causes *B. minutus* to disintegrate and die is a point which has already been made by WIGHT (1926). This I was able readily to confirm, by demonstrating that less than 1 per cent. of bile was necessary to cause more or less instant death and as low a concentration of bile salts as 0.09 per cent. ( $\Delta = 0.0045^\circ \text{C}$ ) was sufficient completely to inhibit growth. Application of Pettenkofer's test to the stools in question showed that a considerable amount of unaltered bile salt was present in these faeces, this alone being amply sufficient to account for the failure of the ciliate to develop in them.

Another point of interest in connection with any coprozoic form is the question of its ability to pass through the alimentary canal in the encysted condition and subsequently to excyst in the faeces after they have been voided. That some coprozoic protozoa (the so-called *Darmpassanten* or gut passers) are able to do this is well known and this faculty has occasionally led to a



diagnosis of coprozoic forms as intestinal parasites on the grounds that the stool sample was taken with all due precautions as to sterility. It was therefore considered advisable to investigate this point in the case of *B. minutum*. Cysts of this ciliate were fed experimentally to mice the faeces of which were subsequently collected and macerated with tap water. However no ciliates were found to develop in the faecal suspension although cysts from the same sample gave rise to a flourishing culture in a control suspension of faeces from the same mice. It seems, therefore that *B. minutum* is incapable of passing through the intestine in the encysted condition but must always contaminate the faeces outside the body. In this respect its behaviour is similar to that of another coprozoic ciliate, *Lemnis pusillus* (cf HOARE, 1927). *B. minutum* must therefore have reached the original stool which was the source of the culture used in this work either (1) by the use of a bedpan in which the stool was collected which had not been sterilized but merely rinsed out with ordinary tap water or (2) by dilution of the faeces after passage with non-sterile tap water or saline or (3) by contamination of the stool sample with air borne cysts during a period of exposure.

In two other papers (WATSON 1940 and in the press) I have discussed in some detail the morphology and bionomics of this interesting organism. It may not be out of place in this connection to refer briefly to some of the work described in these two papers. *B. minutum* which is normally a soil form is peculiar among ciliates in possessing the ability to exist in water so shallow that swimming is impossible, the little creature creeping about and burrowing through the bacterial masses which occur in its normal environment like a tiny amoeba. It is this power of amoeboid movement which enables it to exist in the viscous substance of a stool, where any ordinary ciliate would be immobilized and would speedily succumb. *B. minutum* indeed, is capable of shrivelling up into an anabiotic resting condition without encystation (WATSON 1943), and will immediately resume its activities as soon as the medium is moistened. Thus, when the infected stool eventually dries up the ciliate passes into a state of suspended animation and resumes its activities as soon as the stool becomes scattered over the soil or mingled with sewage. The truly coprozoic forms are therefore necessarily closely related to soil protozoa on the one hand and sewage protozoa on the other but retain their own individual ecological grouping by reason of their ability to live in an environment heavily contaminated with organic matter coupled with the ability to move freely through a semi-solid medium.

Another point of importance in connection with a coprozoic protozoan is the possibility of mistaking it for a genuine parasitic form. There are a number of cases on record when coprozoic ciliates have been described as new intestinal parasites. While the true nature of some of these pseudoparasites has already been established (cf HOARE, 1927) there remain a few doubtful forms which are still regarded as human parasites. Among these *Balantidium*

*minutus* Schaudinn, 1899, is of special interest. Already WIGHT (1926) noted that *Balantrophorus minutus* which occasionally occurs in Californian water supplies, has sometimes been recorded as *Balantidium minutus*. Having made a very careful comparison of the structure of *Balantrophorus minutus* with SCHAUDINN'S original description of his species, I am very strongly of the opinion that he was actually dealing with a stool heavily contaminated with *Balantrophorus minutus*. This question is discussed more fully in another publication (WATSON 1945).

In conclusion it may not be out of place to make some comment on the possible evolutionary significance of the coprozoic protozoa. It is generally accepted that the protozoal inhabitants of the human intestine have evolved from free-living ancestors. Some observers have even gone so far as to suggest that fresh water forms could develop in the human gut if given an opportunity to do so (SCHULZ, 1899 YAKIMOFF 1922). I have been able to show that as far as *Balantrophorus minutus* is concerned this organism is unable to survive for more than a few hours at 37° C. and that cysts which have been exposed to this temperature for a few days are no longer viable. It is therefore clear that if a coprozoic organism is later to take the further evolutionary step of becoming adapted to live in the alimentary canal it must be capable of passing through the gut in the encysted condition of hatching out before reaching the anus and of living and multiplying at the temperature of the body. Although this sequence of events has been observed in certain flagellates (*Copromonas subtilis*) in connection with cold blooded animals, it has never been observed for ciliates and never for any type of protozoon in connection with warm-blooded hosts.

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medical officers of the Government Laboratories and Hospitals Dr K. S. KRIKORIAN Jerusalem, Dr A. MALCHU Bnei Brak and Dr R. REITLER, Haifa.

### SOURCE OF THE BACTERIOPHAGES

Thirteen specific bacteriophages were kindly provided by Col J. S. E. BOYD. Two local bacteriophages were isolated, one from the cesspit of the Hadassah Municipal Hospital in Tel Aviv and the other from the Government Hospital in Bnei Brak by the kind help of Dr GRUSCHKA (Tel Aviv) and Dr. MARBACH (Bnei Brak).

### BIOCHEMICAL TESTS

The fermentation of xylose and arabinose was tested on semi solid media consisting of nutrient agar diluted 1/8, 0.5 per cent. of the carbohydrate to be tested and 1 per cent. of Andrade indicator. The results were read after 3 or 4 days at 37°C. The utilization of d tartrate and citrate was tested on peptone media prepared according to KAUFFMANN (1941). In order to obtain clear-cut results the incubation period was extended to 14 days and only the weak acid acetate precipitation test performed. The utilization of ammonium as a source of nitrogen was tested with and without the presence of  $\text{Na}_2\text{S}_2\text{O}_7$  according to the method of BRAUN and SILBERSTEIN (1943). The solutions used were (expressed in per cent.) sol I:  $\text{NaCl}$  0.5,  $(\text{NH}_4)_2\text{SO}_4$  0.5,  $\text{KH}_2\text{PO}_4$  0.05,  $\text{K}_2\text{HPO}_4$  0.15,  $\text{MgCl}_2$  0.01 and lactate 0.5. sol II: 10 c.c. of a 5 per cent solution of  $\text{Na}_2\text{S}_2\text{O}_7$  were added to 1 litre of sol I. Five subcultures were made in order to establish the ability of a strain to utilize ammonium as a source of nitrogen. The results of the biochemical examination are presented in the following table.

TABLE I  
BIOCHEMICAL TYPES OF *Eberthella typhosa*.

Group.	Number of Strains.	Fermentation of		Utilization of	
		Arabinose	Xylose	Citrate	d Tartrate.
1	111	O	+	+	+
2	70	O	O	+	+
3	3	O	+	+	O
4	1	+	+	+	+
5	1	O	O	O	+

The table shows that the Palestine strains fall into two main biochemical types in addition, five strains fell into three variants. Most of the strains did not utilize ammonium and of those that did only a few grew in the solution without  $\text{Na}_2\text{S}_2\text{O}_7$ . In Group 1 seven strains utilized  $(\text{NH}_4)_2\text{SO}_4$  without and

ten only with  $\text{Na}_2\text{S}_2\text{O}_3$  and in Group 2 the numbers were five and eighteen respectively

The fermentation of the pentoses and utilization of the organic acids were constant properties and the results could be reproduced by repeated examinations of the same strain. On the contrary the utilization of ammonium was not reproducible with the same strains and hence is of no value for purposes of classification.

All strains with one exception utilized citrate if the incubation was extended to 14 days and only three strains did not utilize d tartrate. It is interesting that although four different sources of carbon were tested and therefore sixteen different combinations of reactions were possible only five were obtained

#### SEROLOGICAL EXAMINATIONS

The serological tests were performed with a pure O serum produced by immunizing rabbits with bacteria heated 150 minutes at 100 C and a pure Vi serum produced by absorption of a  $\text{Vi} + \text{H} + \text{O}$  serum with the classical strain H901. The results were similar to those obtained by FELIX, HRIKORIAN and REITLER (1935). Using the terminology of KAUFFMAN (1941) the types were as follows:  $\text{Vi}++9$ ,  $\text{Vi}+148$ ,  $\text{Vi}(\pm)35$ ,  $\text{Vi}03$ . However these results obtained with 24 hour cultures did not show the real Vi potentialities of the strains. We tried to demonstrate the Vi potentialities by two different methods. First by the method of CRAIGIE and BRANDON (1936) with 4 hour broth cultures no marked differences between the O agglutination ability of 4- and 24-hour cultures were observed. Second by enhancing the virulence by mice passages. 0.05 c.c. of broth cultures were injected intraperitoneally and on the next day heart blood was cultured on the surface of an agar slant and the 24-hour growth used for agglutination. The results with 47 strains were as follows:

TABLE II  
SEROLOGICAL TYPES BEFORE AND AFTER MICE PASSAGE

Vi Type Cultures before Mouse Passage		Vi Type Cultures after Mouse Passage			
Number	Types	$\text{Vi}++$	$\text{Vi}+$	$\text{Vi}(\pm)$	O
33	$\text{Vi}+$	20	13	—	—
6	$\text{Vi}(\pm)$	1	4	1	—
6	O	2	1	1	2
47	All types	23	20	2	2

If broth cultures were made from the heart blood cultures and examined after 4 hours incubation then the phenomenon of CRAIGIE and BRANDON (1936) was observed—twelve out of twenty Vi + cultures had lost their agglutinability in the anti-O serum and the total of Vi ++ strains rose to thirty-five out of forty-seven. The same cultures examined after 24 hours incubation had regained their agglutinability in the anti-O serum, and the next subcultures were agglutinable after 4 as well as after 24 hours incubation. The results show that by a single mouse passage a transitory change from Vi + to Vi ++ may be brought about.

#### THE BACTERIOPHAGE TEST

The bacteriophage test was carried out according to CRAIGIE and ILY (1938). Most of the strains were sensitive to bacteriophage C. One of the phages isolated in Palestine the BB-phage (Bnei Brak) acted on the same strains as the C phage while the TA (Telaviv)-phage showed a much wider field of action and influenced strains not attacked by C and BB-phages, as well as those attacked by other specific phages. The BB-phage is, therefore, presumably identical with the C phage, while the TA phage is non specific. We could divide all strains into three groups—those sensitive to phage C (and BB) those sensitive only to the non specific phage TA (imperfect Vi forms) and O forms not affected by any phage.

The relation of the biochemical serological and phage types is shown in the following table.

TABLE III  
RELATION OF BIOCHEMICAL, SEROLOGICAL AND PHAGE TYPES.

Biochemical Group.	Number of Strains.	V Type.				Phage Type.			
		V ++	V +	V (±)	O	C	TA only	O	
1	111	7	64	17	3	104	4	3	
2	79	2	50	18	—	4	5	—	
3	3	—	3	—	—	3	—	—	
4	1	—	1	—	—	1	—	—	
5	1	—	1	—	—	1	—	—	
All groups	195	9	140	35	3	153	9	3	

The question arises whether a given biochemical serological and phage type of the infecting strain is constant or variable. Repeated examinations were carried out with strains from a group of typhoid patients and carriers. The results were as follows—

Case 1—D S. from Moledeth. Urinary carrier. Urine cultures on 18.11.43, 20.11.43 and 23.12.43 and culture from a urinary calculus of 23.12.43. Result: all strains belonged to group 1 phage type C. The serological characters were different: 18.11.43  $V_i$  ++, 20.11.43  $V_i$  ( $\pm$ ), 23.12.43  $V_i$  ++, 28.12.43  $V_i$  +.

Case 2—R. K. from Mishmar Haemek. Typhoid fever. Blood cultures on 17.10.43, 2.12.43 and 20.12.43. All strains group 2, phage type C and  $V_i$  +.

Case 3—T. K., from Kiriat Havim. Faecal carrier. Cultures on 20.10.43, 12.12.43, 20.12.43 and 5.1.44. Result: all strains belonged to group 1 phage type C. The culture on 28.12.43 was  $V_i$  ( $\pm$ ), all the others  $V_i$  ++.

Case 4—J. J. from Haifa. Typhoid fever. Stool cultures on 20.12.43 and 28.12.43 and 10.1.44. All strains belonged to group 1 phage type C  $V_i$  +.

Case 5—B. W. from Zikhron Jakob. Typhoid fever. Stool cultures on 29.11.43 and 8.12.43. Result: group 2 phage type C  $V_i$  ( $\pm$ ). M. W., mother of B. W. typhoid fever blood culture on 2.1.44. Result: group 2, phage type C  $V_i$  +.

Case 6—S. K. from Maso. Typhoid fever. Blood cultures on 8.12.43, 11.12.43 and 18.12.43, stool culture on 8.12.43. All cultures belonged to group 5 phage type C  $V_i$  +.

Case 7—M. K., from Haifa. Faecal carrier. Cultures on 31.10.43, 7.11.43 and 28.11.43. All cultures belonged to group 1 phage type C,  $V_i$  +.

Case 8—A. B. from Kiriat Havim. Typhoid fever. Stool cultures on 28.12.43 and 9.1.44. Group 1 phage type C  $V_i$  ++.

These results show that there are differences in the degree of development of  $V_i$  antigen in the strains isolated from the same patient, while the phage type and the biochemical properties (behaviour on xylose, arabinose, citrate and d tartrate) were unchanged.

### THE LOCAL DISTRIBUTION OF THE BIOCHEMICAL TYPES

The following table shows the distribution of the different biochemical types in the main cities and their surroundings.

TABLE IV  
THE DISTRIBUTION OF THE BIOCHEMICAL TYPES.

District of Isolation	Biochemical Group.					Xylose A.	Xylose O.	All Types.
	1	2	3	4	5			
Jerusalem	33	19	—	—	—	33	19	52
Telaviv and Jaffa	37	20	1	—	—	38	29	67
Haifa	41	31	2	1	1	44	3*	78
All places	111	70	3	1	1	115	50	195

The table shows that the main types show the same distribution in the whole country, while the rare types 3, 4 and 5 were found almost only in Haifa. Furthermore, there is a high percentage of xylose negative strains: Jerusalem 36.5%, Telaviv/Jaffa, 43.2% and Haifa, 42.1% per cent. Average 41% per cent.

Another question is the appearance of the different types in local outbreaks of typhoid fever. During 1943 there occurred outbreaks of typhoid fever in several agricultural settlements. The settlers lived in individual houses but ate in a common dining room. In Revivim, near Rishon le Zion, there were nineteen cases among sixty five people. Four strains were examined and all belonged to group 1 phage type C Vi +. In Noar Agudathu there were four cases among sixty men. Three strains were examined two belonged to group 2 and one to group 1. In Maos there was an outbreak of thirty-one cases among 235 persons. The first case S. K. (No. 6 above), was examined repeatedly during December 1943 and found to belong to group 5. He arrived ill from Turkey. During January 1944 the outbreak of thirty cases occurred. (Information from Professor W. STRAUSS Hygiene Service, Hadassah Medical Organization.) The twenty two cultures isolated from twenty cases belonged to group 2, phage type C. The cultures from twenty cases gave typical growth of dwarf forms, while those from S. K. gave normal colonies on agar. It should be recorded that the original dwarf forms belonged either to the Vi ( $\pm$ ) or O type and were lysed only by the non specific TA phage but after a single passage through plain broth the strains were sensitive to the specific C-phage and became Vi +. Similar dwarf forms were isolated by HIRSHI (1939) during an epidemic outbreak in a neighbouring place (Masad).

The results of typing in the three outbreaks suggest that there may be some importance in establishing whether one or more biochemical types occur in an epidemic. At Revivim all strains belonged to group 1 and it seems certain that there was a common source of infection. In Noar Agudathu there were only four cases during a month and two different types were isolated. In Maos it could be shown that the outbreak in January was caused by the same strain (group 2) and that it differed from that in the suspected primary case (S. K.) which appeared in December.

#### *Distribution of the Types in the Various Sectors of the Population*

Of fifty two strains isolated in Haifa, thirty four cultures came from Jews and eighteen from Arabs. The xylose negative strains were thirteen Jewish (38.2 per cent.) and seven Arab (38.9 per cent.) It seems, therefore, that the distribution of typhoid strains in Haifa and its surroundings is the same in both communities.

#### DISCUSSION

The results reported above show that by combination of biochemical and phage tests it is possible to obtain a differentiation of the typhoid strains, which may have epidemiological value. The advantage of such a combined examination is particularly obvious where as in the strains studied, only one phage type is prevalent (183 out of 195 cases). This combination is particularly important

because the phage type is independent of the biochemical behaviour of the strain and consequently it is possible to split up the same phage type into biochemical subgroups. The few examples given show that these properties are constant a fact which was observed by KRISTENSEN (1938). This constancy enables us to recognize the same causative agent in the course of an epidemic outbreak.

The typing of *Eberthella typhosa* by the combined methods may serve to clear up some points of the epidemiological question in the country. In a country limited in its area such as Palestine, where typhoid fever is endemic, it would be expected that only few types of *E. typhosa* would exist and that these would be distributed in a more or less equal proportion in different parts of the country and among the different sectors of the population. The results of the examinations are in accord with this expectation.

Comparing the results with those reported from other countries we find that the distribution of types in Palestine is different. It is therefore hard to believe that typhoid fever is not endemic here, but is imported from other countries. The reports from the European Continent are not available, but in England FELIX (1943) found at least thirteen phage types with only forty-four out of 440 belonging to type C. BOYD (1943), in his work on Axis prisoners from the Western Desert, found type C together with four other types, and sixty five out of 256 strains belonged to the C type. LAZARUS in the United States (1941) found the C type in forty cases out of 377 together with eleven other types. In Peiping, YEN (1939) did not find any type C strains, but five other types were present.

The high percentage of xylose negative strains (41 per cent.) is an unexpected result and does not agree with the findings in other countries. Thus HARTOCH, SCHLOSSBERGER and JOFFE (1926) examined seventy seven strains from Leningrad and found only twenty two xylose negative, i.e. 26 per cent. HIRSZFELD, AMZEL and ROSENBERG (1933) in Poland examined 569 strains and found seventy-one negative i.e. 12.5 per cent. SCHIFF (1929) found in Prussia 8.1 per cent. and in the southern districts of Germany 37 per cent. SILBERSTEIN found in Berlin 13 per cent., VATER in southern Germany 21 per cent. It seems, therefore, that from no country has so high a percentage of xylose-negative strains been reported as in Palestine.

There is a further question which seems to deserve attention. During the last 2 years there was a marked increase in the severity of typhoid cases in Palestine, and it was not clear whether this was due to a change of the virulence of the infecting strains or to an increased susceptibility of the infected individual. Our results show clearly that since 1934 the year of the examination by FELIX, KRIKORIAN and REITLER, there were no changes in the Vi type of the infecting strains. As in 1934 most of the strains isolated 1940 to 1942 and 1943 belonged to the intermediate WV type. It seems, therefore, that the increased severity is probably not due to the strain but to the diminished host resistance.



## SUMMARY

1 One hundred and ninety five strains of *Eberthella typhosa*, isolated from different sources and various places in Palestine were examined by biochemical and serological methods and by phage typing

Most of the strains belonged to the WV type. With few exceptions all strains belonged to phage type C.

2 Two local phages were isolated one of them (BB) showed the same behaviour as a C-phage the other (TA) was a non specific bacteriophage.

3 By fermentation of arabinose and xylose and the utilization of citrate and d tartrate two major and three rare subgroups were differentiated.

4 By repeated examinations of patients and carriers it could be demonstrated that the behaviour of the strains towards the pentoses and organic acids does not undergo any change.

5 The distribution of the different types of *Eberthella typhosa* in Palestine is nearly the same in all places and communities of the country. Marked seasonal differences were not observed.

The authors gratefully acknowledge the help and co-operation of all those who provided the cultures and bacteriophages and gave the necessary information.

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## CORRESPONDENCE

### AMOEBIASIS

*To the Editor TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR

The admirable paper by Dr ADAMS\* in these TRANSACTIONS has just reached me. Between June and October 1944 one examined a number of officers before their return to duty after sick leave in Kashmir.

The most striking impression received was the sharp contrast between sick and wounded. To say the wounded were exalted the sick depressed, would scarcely exaggerate the sharpness of this distinction. The wounded appeared physically brisk, mentally alert. The sick appeared physically slack, mentally dull.

Except for some in this subgroup whose mind's eye was obviously fixed on colon and stools, about which they were voluble, mental inertia was perhaps most striking in the subgroup of sick who had been treated for amoebic dysentery.

Were these men sick because of their physical and mental depression or was their physical and mental depression the result of amoebiasis or its treatment? In considering this question the statement of CLARK† that the effective therapeutic dose approaches the toxic dose is suggestive, particularly where he quotes DOBELL and BISHOP's finding in 1929 that in monkeys the curative dose of emetine produced toxic symptoms in 80 per cent. of cases.

What are the factors on which depend the grading of a population into those who are negative to examination—those (about 8 to 10 per cent.) who without symptoms are reported to pass E.H. cysts—those with signs and symptoms of dysentery, hepatitis or liver abscess?

If as Dr HOARE said WALKER established in 1913 that the pathogenicity of *E. histolytica* depends rather on human susceptibility than on difference in the virulence of the parasite strain, in what does human susceptibility consist?

\* ADAMS, A. R. D. (1945) Amoebiasis with special reference to treatment. *Trans. R. Soc. trop. Med. Hyg.* 38 (4) 237.

† CLARK, A. J. (1937) *Applied Pharmacology*, 6th Ed. London: Churchill, pp. 660-662.

Is there an objective quantitative measure of human susceptibility by which a population could be classified as above independently of the results of clinical or microscopical examination?

And if as Dr LOURIE said, HALAWANT showed in 1930 that acquirement of emetine resistance by *E. histolytica* is no myth why is our treatment still aimed not once but repeatedly rather against the parasite than at man's susceptibility or more recently against the parasite and for his colon, rather than at his susceptibility?

CLARK again states "treatment by full course of hypodermic injections cures about 30 per cent of chronic cases." Is emetine resistance the cause of the failure to be expected in 70 per cent. of cases treated?

Anyone who compares the records of the health of the Army in India for the two periods, roughly 1922 to 1927 and 1928 to 1933 would find a *ratio* face in the proportion within the dysentery diarrhoea, colitis group recorded as amoebic. Preponderance of amoebic in the first of bacillary type in the second period is unlikely to be a correct estimate. If bacillary were in fact preponderant in both periods many suffering from bacillary would have been treated for amoebic dysentery in the first. Is the preponderant diagnosis in each period due to consensus of opinion amongst independent observers or to *ex cathedra* suggestion?

Is the proportion of amoebic to bacillary dysentery exaggerated in this war as it was shown to be in several theatres in the last?

When one considers these questions one finds oneself almost as ignorant in 1945 as one was when one began to use emetine hydrochloride in 1917 when hopes were high.

Dr ADAMS's frank paper and the discussion upon it suggest that I am not alone at least in my dissatisfaction with the gaps in our knowledge, with the results of our treatment. But dissatisfaction is not enough unless it becomes sufficiently active to press home on those who can organize it, the urgent need for the study of amoebiasis by groups of first class bacteriologists, biochemists, clinicians, pharmacologists, protozoologists and statisticians. The sooner these are got together the sooner they set to work, the better for the large number of unfortunate men and women who, during the coming years, will need treatment for their chronic physical and mental disability. One cannot end better than by quoting the words of DIXON\* therefore there must be other factors than amoeba and emetine to consider

I am, etc.,

J B DE W MOLONY

*Lt-Col., I.M.S. (retd.)*

Edinburgh.

\* DIXON W. E. (1925) *Manual of Pharmacology* 6th Ed. London Arnold  
p 290

*The previous number of these Transactions Vol XXXIX No 2  
was published on October 29th, 1945*

# TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

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VOL. XXXIX. No 3. DECEMBER 1945

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OPENING MEETING OF THE THIRTY-NINTH SESSION,

held at

Manson House, 28 Portland Place, London, W ,

on

Thursday, 18th October, 1945, at 8 p m

THE PRESIDENT

C M WENYON C.M.G. C.B.E. M.B. B.Sc. F.R.S.,  
in the Chair

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## PRESIDENTIAL ADDRESS

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TROPICAL MEDICINE IN WAR AND PEACE

BY

C. M. WENYON

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It occurred to me in trying to think of a suitable subject for my Presidential Address, that it might be appropriate, now that the war is over to review some of the more important advances which have been made in tropical medicine during its progress

The need for maintaining the health of the large numbers of men that it was necessary to send to tropical and sub-tropical countries was a great stimulus to research into the possibilities of improvement in the prevention and treatment of those diseases which are particularly prevalent in these areas. A great deal has been done under the general heading of hygiene and sanitation, which includes feeding clothing and housing and one is sometimes led to wonder if the low incidence of certain food and water-borne diseases may not be due to these measures rather than to some of the protective inoculations to

which the troops had to be subjected. I think it can safely be stated that never before have troops been called upon to campaign in tropical lands in greater comfort, or perhaps I should say in less discomfort, than in this war.

### TROPICAL MEDICINE IN THE LAST WAR.

Many of us here have had the experience of two wars, and some of us, a doubt, of other wars as well. I served in the 1914-1918 war and saw something of medical progress during its 4 or 5 years, and in thinking of the subject at which I have chosen to address you I was led to wonder what outstanding advances in tropical medicine had been made during that period.

Many of these, it appears to me, were more of a negative than of a positive nature. Thus, it was finally realized that quinine was not such a good drug as we had supposed it to be. Even in maximal daily doses it failed to prevent attacks of malaria in a large proportion of those taking it, though it is my belief that under prophylactic quinine these attacks were fewer and generally less severe than they otherwise would have been. When quinine was given in larger doses for treatment of malarial attacks there is no doubt that the fever was controlled and parasites banished from the blood. Unfortunately relapses were very common and the interesting observation was made that one diagnosed as malignant tertian malaria and treated as such, frequently had relapses of benign tertian malaria. So striking was this that the suggestion was made on more than one occasion that *Plasmodium falciparum* and *P. vivax* were merely different phases of one species. Those who made this suggestion failed to realize that originally both infections had been present, though only one had been detected because of the tendency of the malignant tertian parasite to keep in abeyance that of benign tertian malaria. Quinine had cured the one infection but not the other which asserted itself later on.

In the case of the dysenteries which were such a serious cause of sickness there gradually came about a realization that emetine, to which we had pinned our faith, too often failed in the chronic amoebic infections. To overcome this difficulty emetine bismuth iodide was introduced. Given by the mouth, it appeared to bring about a greater percentage of permanent cures than other methods of emetine administration. As regards bacillary dysentery no great improvement in treatment occurred though this was badly needed. Even amoebic dysentery serum, held in high esteem by some physicians, really appeared to have little if any influence on the disease.

Perhaps I might mention here the confusion which existed in the early days of the last war between amoebic and bacillary dysentery—a confusion which resulted in a diagnosis of amoebic dysentery being made in nearly all cases of dysentery contracted in Gallipoli, in Egypt and elsewhere. It gradually came to be realized that the bulk of the cases were bacillary in nature and that only a small percentage, under 10 per cent., were of amoebic origin. This confusion led to a wholesale and unnecessary administration of emetine.

While on the subject of amoebic infections I am reminded that it was during the last war that it was first discovered how widespread amoebic infection was amongst apparently healthy individuals. So extensive was it amongst the troops that any idea that may at first have been held of attempting to identify every carrier and ridding him of his infection by injections of emetine—then supposed to be the infallible remedy—had to be abandoned. As only a relatively small percentage of those harbouring the dysentery amoeba developed actual dysentery the policy was adopted of dealing only with those showing clinical signs of their infection. In connection with the study of amoebic infections a great deal of information was acquired regarding the intestinal protozoa of man in general.

During the last war one of the most striking advances in tropical medicine was the solution of the bilharzia problem in Egypt. It was shortly before the war that Japanese helminthologists had given a complete account of the life history of *Schistosoma japonicum*. In what was then a dramatic announcement they showed how they had been successful in infecting snails in the laboratory by exposing them to the miracidia which hatched from eggs introduced into water—how in due course cercariae with bifid tails emerged from the snails and how small laboratory animals immersed for a short time in the water containing the free-swimming cercariae contracted a schistosome infection. Soon after this announcement the London School of Tropical Medicine arranged for Professor LEIPER to go out East to examine these claims. He visited the Japanese workers who showed him their experiments, which he was able to repeat. The war had then started and Professor LEIPER realized the importance of schistosomiasis to our troops in Egypt. He was dispatched to Egypt by the War Office and there, applying the Japanese technique to both *S. haematobium* and *S. mansoni* he and his co-workers quickly showed that the method of infection was identical with that of *S. japonicum* and that contrary to the claims of the German helminthologist LOOSS who had studied the subject for many years in Egypt, snail intermediate hosts were involved in the life cycles and that infection resulted from bathing or wading in water in which cercariae emerging from these snails were swimming.

Another subject which received much attention in the last war was Weil's disease. It is not strictly a tropical disease but being a leptospiraemia it is closely allied to several similar infections in outbreaks in the tropics. Much information was accumulated amongst the troops in France. It is interesting to note that this disease had been thoroughly studied by the Japanese before the war.

During the war active research had been carried out on typhus fever in several countries. The chief outcome of this was the final demonstration that the louse was the vector of European typhus. Furthermore WEILL and FELIX first isolated *Bacillus proteus* from the urine and blood of cases of typhus and showed that it gave a specific agglutination reaction with the serum of typhus patients—a reaction which could be employed as a procedure for the

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agreed that one pill of the drug or a tenth of a gramme three times a day for a week, was sufficient in nearly all cases to reduce the fever and clear the blood of parasites. It was recognized that in the case of *P. falciparum* infections this treatment in many cases brought about a complete cure not followed by relapse whereas in the case of benign tertian malaria relapses commonly occurred. Also as a prophylactic atabrin had been extensively used, and reports appeared to show that it was superior to quinine. The drug was given in doses of 0.2 or 0.3 gramme once or twice a week, or in a daily dose of 0.05 gramme. In some cases a daily dose of 0.1 gramme had been employed. It was fairly well agreed that atabrin was very effective, and though it produced the yellow staining of the skin it could be taken daily over long periods sometimes amounting to several years it was stated, without any harmful effects. In spite of these reports there was considerable hesitation as to the advisability of accepting atabrin as the desired substitute for quinine. It was thought that the evidence that atabrin could be taken regularly over long periods without undermining the health through injury to the liver or other cause was inconclusive. However this may be, reliance had to be placed at first on what quinine supplies there were while field trials of atabrin were being carried out in Africa. In some of these the drug appeared to be toxic but, on the whole reports were favourable, so much so that both in this country and the United States strenuous efforts were made by chemists to discover a method of preparing it on a large scale. These were highly successful so that finally adequate supplies were forthcoming. It was still uncertain how effective atabrin was either for treatment or prophylaxis and quinine had still its advocates. Matters were in this somewhat unsatisfactory state when Brigadier FAIRLEY then with the Australian Army in the South West Pacific, realized that it was imperative to obtain more precise information about the properties of atabrin and other possible malaria remedies. Accordingly the Experimental Malaria Centre was set up at Cairns, in Australia, under his direction, and there volunteers under strict control were infected with malaria by means of mosquitoes imported by air from New Guinea while atabrin quinine and other drugs were administered for varying periods and in varying doses before and after exposure to infection. Blood and other examinations were regularly carried out and the presence or absence of infection was often determined by the injection of 250 c.c. or more of blood into fresh volunteers. Frequently groups of men on prophylactic drug treatment were submitted to violent exercises and exposures with a view to simulating strenuous active service conditions in order to see if attacks of malaria could be induced. It is impossible for me to give any further details of the painstaking method of these investigations carried out by Brigadier FAIRLEY and his team at Cairns in Australia. Those of us who had the privilege of hearing him lecture on the results of this work before this Society during his visit to this country last January will have realized with what scientific thoroughness all the experiments were carried out. The main outcome of this work was the clear demonstration that persons taking 0.1 gramme of atabrin that is



one tablet a day did not suffer from malaria, though exposed under active service conditions to the most intensive infection by the bites of mosquitoes, and that the daily dose could be continued practically indefinitely with no more inconvenience to the individual than the slight yellow coloration of the skin. It is not too much to say that this demonstration, which convinced even the most sceptical of military commanders altered the whole course of the war in the Pacific. Before this the incidence of malaria was terrific (750 per 1 000 per annum) but with the adoption of the one pill of atabrin a day under strict military discipline the incidence fell rapidly to 26 per 1 000 so that malaria became almost a negligible problem as regards troops in the field. In other localities where malaria is hyperendemic, such as West Africa and Burma, the enforcement of the one pill a day rule under strict military discipline has been followed by similar reductions in the incidence of malaria. This indicates that the Pacific strains of malaria are no more susceptible to the action of atabrin than are those of some other countries. Whether there exist strains more resistant to atabrin cannot be stated till a greater number have been tested. Experiments at Cairns clearly demonstrated that quinine in a daily dose of 5 or even 10 grains a day is incapable of preventing attacks of malignant tertian or benign tertian malaria with any degree of certainty. Though atabrin is better than quinine it has its limitations, as relapses are not infrequent after its use. In dealing with troops returning from malarious areas in New Guinea the procedure was adopted of continuing the daily dose for about 3 weeks after the malarious area had been left. In spite of this, within a few weeks of ceasing to take the drug attacks of malaria occurred. These attacks, however were invariably due to benign tertian malaria—the daily dose of atabrin had entirely eliminated any malignant tertian infection which had been contracted. The selective action of atabrin was amply confirmed by controlled experiments carried out at Cairns. It is in some respects similar to the experience of the last war to which I have already referred, the cases of malignant tertian malaria which had been treated with quinine in Macedonia and presumably cured, after return to England suffered relapses not of malignant tertian but of benign tertian malaria. Though atabrin fails to cure benign tertian malaria entirely the demonstration that it is an effective prophylactic—even in areas where the infection rate by the bites of mosquitoes is very high indeed—is one of the outstanding achievements of the war. While taking one pill a day a man can carry on intensive warfare with little fear of being incapacitated by attacks of malaria. It seems that the loss to the Japanese of the main cinchona plantations of the world was a blessing in disguise, for it was the chief stimulus which led to the production of atabrin on a large scale. As Sir LEONARD ROGERS remarked at a meeting of this Society it is paradoxical that a German invented drug was one of the chief means of our defeating their ally the Japanese. Someone who ought to know said to me recently that without the use of atabrin in Burma it would have been impossible to have prevented the Japanese from over-running India.

## MALARIA—EXOERYTHROCYTIC DEVELOPMENT

Some significant observations were made at Cairns on the infectivity of the blood taken from volunteers on whom infected mosquitoes had been allowed to feed. Blood taken from one arm 7 minutes after mosquitoes had fed on the other arm was infective to fresh volunteers showing that sporozoites were circulating in the blood. After 30 minutes the blood was no longer infective and it remained so in the case of *P. falciparum* till the 7th day when it again became infective and in the case of *P. vivax* till the 9th day. When the experiment was repeated on volunteers taking one pill of atebirin a day the blood in the case of *P. falciparum* infections was again negative till the 7th day when it became infective though parasites could not be microscopically demonstrated in thick films. It remained infective on the 8th and 9th days and then became persistently negative if the daily dose of atebirin was continued for the prescribed period of about 3 weeks. In the case of *P. vivax* infections on the other hand, a period of non infectivity of the blood was almost invariably followed by attacks of malaria some time after the daily dose of atebirin had been stopped. The general result of these observations is that after an infective mosquito bite sporozoites are present in the blood for a few minutes only the blood is then negative for 6 to 9 days when it again becomes positive. If atebirin is being taken daily this positive phase still occurs but it lasts only 2 or 3 days in the case of *P. falciparum* infections. It would seem that the only reasonable explanation of these facts is that the malarial parasites during the negative blood phases are undergoing development in certain cells of the body other than red blood corpuscles and that atebirin has no action on these stages or fails to reach them. As atebirin cures malignant tertian malaria entirely it might be supposed that the duration of this phase in the case of *P. falciparum* is a short one and that as it fails to cure benign tertian malaria entirely this phase of *P. vivax* persists for long periods and is responsible for the many relapses which are characteristic of this type of the disease. Unfortunately as regards human malaria, these stages are as yet hypothetical, as no one has seen them, though efforts are being made in several countries to discover them if they exist. To my mind the various reports which have appeared of the discovery of such forms in human malaria are quite unconvincing.

Another important advance in the field of malaria has to do with the development of sporozoites of *Plasmodium gallinaceum* of chickens. As you know this was the parasite in which JAMES and TATE discovered the remarkable exoerythrocytic stages of development. As mosquitoes are easily infected with it, it seemed particularly suitable for the study of the development of sporozoites in fowls. A number of observers have attempted to trace this development with varying success. Of the earlier workers REICHENOW and MUDROW in Germany and PARANESE in South America were the most successful but it was left to HUFF and COULSTON to give the most complete account in their paper published at the end of 1944 of what happens to the sporozoite when it is injected into the chicken. They have found that in the skin the sporozoites

quickly penetrate the mononuclear phagocytic cells of the connective tissue. There they increase in size and in about 42 hours break up into 100 to 200 merozoites when the cryptozoic generation is completed. These cryptozoic merozoites enter other cells, either local or more remote and commence the first metacryptozoic generation. This is very similar to the first or cryptozoic generation and, like it, ends in about 42 hours with the production of the first metacryptozoic merozoites. These merozoites are scattered far and wide throughout the body and a few apparently enter red blood corpuscles and start the erythrocytic phase of development. Others enter macrophages or endothelial cells of various organs to commence the second metacryptozoic generation. Shortly before this it has become possible to distinguish two types of mature schizont, or segmenter as the authors call them. One produces from 100 to 200 large merozoites and the other from 500 to 1,000 small merozoites. They are termed macro- and micro-merozoites respectively. As development proceeds, more of the latter and fewer of the former are produced, while with each succeeding metacryptozoic cycle larger numbers of the merozoites enter the red blood corpuscles. It may be that the change in type of the schizont is correlated with the increased invasion of the red blood corpuscles which takes place.

Now that the early stages of development of the malarial parasite of fowls has been so clearly demonstrated it is very tempting to suppose that a similar development exists in the case of the human malarial parasites. HUFF and COULSON have been careful to point out that the early stages of development have only been seen in those bird malarial parasites in which exoerythrocytic forms are common. They note that in many bird malarial parasites such forms have never been found though repeatedly looked for. They think it likely that some species of malarial parasite undergo their cryptozoic development in cells which are different from those utilized by the parasite of fowls. They also think that the possibility of a direct development of a sporozoite into a parasite of a red blood corpuscle (as SCHAUDINN so long ago described) cannot be entirely ruled out, though there is much indirect evidence against the hypothesis. As we have already noted, Brigadier FAIRLEY and his co-workers at Cairns have shown that, following injection of sporozoites into human volunteers, the blood, after being infective to other volunteers for a few minutes, then becomes non infective for a week or more when it once again becomes infective. This is highly suggestive of a development in some part of the body other than in the red blood corpuscles. In this connection I was reminded a short time ago by Sir PHILIP MANSON BAKER of a paper which I had forgotten. It was on the subject of malignant malaria in Macedonia, by GARDNER and MILLAR, published in 1920. One section of the paper was headed "The hiding places of the malarial parasites in the body" which shows that even then the possibility of such hiding places was being considered. They stated that they had seen malarial parasites in the trabeculae and supporting cells of the spleen.

pulp in stellate cells in the liver and in the heart in lymph spaces between the muscle fibres and, most remarkable of all, in the sarcoplasm which immediately surrounds the nuclei of the muscle fibres themselves. These forms contained no pigment but were small solid bodies with a single granule interpreted as chromatin. The authors referred to them as filled in rings. What to me seems most questionable about them is that wherever they were found they were all in the same stage of development. However this may be, the search for the hiding places of the human and monkey malarial parasites is still going on and if such exist there is no doubt that before long they will be brought to light. The important work of HUFF and COULSON will do much to facilitate this quest.

#### INSECTICIDES AND REPELLENTS

While on the subject of malaria and its suppression or prophylaxis we naturally think of the very important discoveries which have been made in the direction of mosquito destruction and the prevention of their bites. The most important relates to DDT an up to-date account of which was given before this Society last February in a most instructive address by Professor BUXTON. The far-reaching applications of this very remarkable substance are likely to play an important part in the prevention of those tropical diseases for which insect vectors are responsible. The employment of DDT in sprays to kill adult mosquitoes and house flies in houses and enclosed spaces in sprays to destroy mosquito larvae in water in dusting powders against lice in solutions for impregnation of clothing and bedding to render them louse-proof are but a few of these applications. I hear rumours of the use of DDT against fleas and tsetse flies, but as far as my information goes it is too early to make any definite pronouncement under these headings. No doubt in due time DDT will be tried out against sandflies and other vectors or their larvae, as a means of prevention of many tropical diseases.

As regards malarial prevention I have seen a recent report by SIMMONS from the United States in which it is shown that the spraying of a room with DDT, using 20 mg per square foot, will cause the death of 60 to 90 per cent. of all wild mosquitoes which enter the room 20 weeks later while the spraying of water using 1/10 lb of DDT per acre, will kill all mosquito larvae, and this at one-fifth the cost of the amount of oil necessary to give the same result. Already DDT has its competitor in the gamma isomer of benzenehexachloride (666) or Gammexane recently introduced by the ICI.

The question of insect repellents is one which has occupied the attention of investigators in this war particularly from the point of view of the louse and the mosquito. This work was commenced in the Entomological Department of the London School of Hygiene and Tropical Medicine under Professor BUXTON. Sir RICKARD CHRISTOPHERS then took up the subject and has been responsible for the major part of what we now know under this heading. A great

deal has also been done in the United States and in Russia. The results of all this work are not yet public property but we do know that the phthalates—dimethyl diethyl and dibutyl phthalate—are very much disliked by many insects so much so that dimethyl phthalate has been employed very extensively for rubbing on the skin to ward off biting insects particularly mosquitoes. It is a clear liquid, slightly thinner than glycerine is practically without smell and is not irritating to the skin. Unlike many other repellents, its effect persists for some hours even in hot countries where men perspire freely. It is effective in repelling the mite vector of scrub typhus, but the butyl phthalate is preferred by Australian authorities for this purpose as it is more effective than the methyl derivative, killing as well as repelling when applied to clothes and bedding. No doubt we shall hear more about these repellents and others, such as the hexanediols which some think are even better when the general release of information takes place. It is quite clear however that for the first time repellents have been discovered which actually do what is claimed of them. They repel insects when applied to the skin, drive them away or even kill them when introduced into clothes and bedding and when sprayed on to mosquito netting as observed by American and Russian investigators will set up a barrier which insects are reluctant to pass even though the mesh is wide enough to admit them easily.

#### TYPHUS.

I have just mentioned scrub typhus, which has threatened our troops in Burma and in the islands of the South West Pacific. This is the Japanese river fever or tsutsugamushi disease, which had been thoroughly studied in Japan long before the war and later in the Malay States. The vector being a mite, is a most insidious creature which it is practically impossible to avoid in those localities where it occurs in abundance. As I have said, some protection is given by suitable clothing and by repellents but these have their limitations. As a result of intensive investigations in this country and in the United States, a formalinized vaccine was prepared from the lungs of nasally infected cotton rats. This gave some protection in laboratory animals and so urgent was the need that it was decided, admittedly on somewhat incomplete evidence, to use it on a large scale in Burma. It seems that the sudden termination of hostilities will have interrupted the large scale experiment which was just commencing.

Louse borne typhus has also been a menace in North Africa, Iraq, Persia and other places. The disease has run its accustomed course and attempts at protection by vaccination have been carried out on a fairly large scale. The vaccine employed has been a concentrated formalinized rickettsial vaccine prepared from egg yolk cultures by the processes of COX and CRAIGIE. Though the full results have not yet been assessed, I believe the general impression is that some degree of protection has been conferred.

In the prevention of typhus there can be no doubt that DDT has given the most remarkable results. Apart from the fact that the dusting of individuals already lousy will quickly clear them, the observation that garments can be rendered louse-proof by impregnation with this insecticide has been of the utmost service in the prevention of typhus and other louse-borne infections. A soldier's shirt once impregnated will retain its insecticidal properties through a number of launderings—practically during the whole life of the shirt. If this were the only property of DDT its discovery might rightly be regarded as one of the most important hygienic advances of the war.

In connection with typhus a great deal of time has been devoted to the study of the antigenic structure of the various typhus rickettsiae. These have yielded very important theoretical results upon which the principle of immunization by vaccination was based. Efforts have been made to improve complement fixation and agglutination tests using rickettsia as antigen but they have not contributed much to the routine diagnosis of typhus which is still based on the old Weil-Felix reaction.

#### DYSENTERY—BACILLARY

I come now to a consideration of the dysenteries of which as in the previous war the bacillary type promised to be the most important. In fact, during the first year or two it seemed to be just as prevalent and there was as usual, every gradation of severity between the serious Shiga cases and the mild forms of gippy tummy. Treatment was by salines and serum but I do not think anyone had much confidence in the latter. Neither could COMPTON convince the majority of those who had the handling of dysentery cases in Egypt that the phage treatment he advocated gave the good results he claimed. Matters were in this somewhat unsatisfactory state when there appeared the papers by MARSHALL and his co-workers of Johns Hopkins University announcing a new sulphonamide, namely sulphanilylguanidine, or sulphaguanidine, which though soluble in water was not so readily absorbed from the intestine as some of the other derivatives so that saturation of the intestinal contents could be obtained. Hoping that on this account the drug would be of use in the treatment of bacillary dysentery MARSHALL and his co-workers tried it on a number of acute Flexner and Sonne infections in many cases the result was dramatic and immediate recovery took place. The drug proved to be not toxic even in large doses and as the blood concentration was relatively low there was less danger of the kidney damage which is associated with some of the other more readily absorbed sulphonamides. These preliminary results were confirmed in Egypt by FAIRLEY and BORD and BUTTLE, who had received a small supply from the U.S. and soon demands for the drug came in from many theatres of war. A period had to elapse before supplies became adequate and efforts were made meanwhile to improve the anti-Shiga serum. A concentrated serum was in process of trial and had been favourably reported upon when the supplies

of sulphaguanidine became sufficient for general use. The results of treatment of bacillary dysentery and of what might be termed the pre-dysentery diarrhoea were so satisfactory that the opinion has been expressed that bacillary dysentery ceased to be a war problem. Other sulphonamides are known to exert a favourable action on bacillary dysentery and it seems probable that sulphaguanidine may not be the last word in the treatment of this disease by the sulphonamide series of drugs. Some claim that succinylsulphathiazole, or sulphasuxidine for short, is better as not more than 5 per cent. of the dose is absorbed from the intestine, as against the 30 to 60 per cent. of that of sulphaguanidine.

#### DYSENTERY-AMOEBC.

At the outbreak of this war amoebic dysentery was very much in the same position as it was after the last war. It was known that emetine hydrochloride by injection would control the acute dysenteric condition and that it also was effective for amoebic hepatitis and liver abscess. In the more chronic cases, however, it too often failed to get rid of the amoebic infection. Better results were obtained with emetine bismuth iodide especially in healthy or relatively healthy carriers, but even this drug might be inactive in those chronic cases where there had been a long-standing colitis. A number of other remedies were tried. These fall into two groups—the arsenicals, such as stovaine and carbasone and the iodo-compounds, like chinofon or yatren diodoquin and vioform. Diodoquin, with a high iodine content and low toxicity has been increasingly used in the United States where it was first introduced in 1906. Lately it has been employed in this country. It is said with some success. As pointed out by MORTON it is possible by the use of these various remedies without placing absolute reliance on any one, to cure a large number of these cases. There still remain far too many which appear to be absolutely resistant and it is in connection with these that one of the most important developments in regard to the treatment of amoebic infections has been made. Some of you will remember that at a meeting of this Society last November Lt-Col HARGREAVES reported that certain cases which were resisting all anti-amoebic treatment and were steadily going down hill improved dramatically when given a course of penicillin. The amoebic infection, however, persisted and it appeared that the improvement was due to the elimination of bacterial infections. The striking observation was then made that after this improvement the amoebic infection responded readily to the routine anti-amoebic treatment. Colonel HARGREAVES has continued his observations along these lines and finds that the initial improvement may be even greater if in addition to penicillin, one of the sulphonamides is also given. An important outcome of this work is that it disproves entirely the view persistently held by some that such cases are resistant in the first place because the amoebae are emetine-fast. No one has yet produced satisfactory evidence that emetine-fast strains exist.

## LEISHMANIASIS

During this war but not perhaps as the result of a particular war effort in this field, a very important investigation which has been going on in India for over 20 years was brought to a successful completion. I refer to the final experiments which have proved that kala-azar in India is transmitted by the bite of the sandfly *Phlebotomus argentipes*. At first the sandfly was incriminated on epidemiological grounds then it was shown that development of the parasite took place in the stomach and proboscis of the fly. This was followed by the discovery of a method of feeding and keeping alive the infected flies which led to the successful transmission of the infection by their bites to hamsters. Finally there was the human experiment in which infected sandflies were allowed to feed on five volunteers from an area where kala azar did not occur with the result that all five volunteers contracted the disease. The whole of this investigation occupying as I have said, over 20 years is a wonderful illustration of team work in which many distinguished Fellows of this Society and others have played a part. In May last we had the privilege of hearing an address on Recent Research on Kala azar in India by Colonel SHORTT who had just returned from India where he had worked for many years on this subject and had recently taken part in carrying out and arranging the final crucial experiment.

Another interesting development in our knowledge of leishmaniasis is due to investigations carried out by Russian workers LATYSHEV and KRIUKOVA in Middle Asia. In certain rural settlements bordering on the desert the incidence of oriental sore is very high. The sandflies responsible for its transmission are *Phlebotomus papatasi* and *P. caucasicus* which breed exclusively in the burrows of gerbils and sousliks. Examination of these rodents showed that 30 per cent. of one of the gerbils *Rhombomys opimus* were infected with leishmania, most of the positive animals having sores on the ears. Furthermore, sandflies were not only found naturally infected but were readily infected by feeding on infected gerbils. It was proved that the gerbils were acting as reservoirs of the human virus which was transmitted to man chiefly by *Phlebotomus papatasi* the other sandfly *Phlebotomus caucasicus* being responsible for maintaining the infection in the rodent population in the burrows. It was found that within the burrows atmospheric conditions remained constant throughout the year so that amongst the rodents there was not the seasonal incidence which characterized the infection in human beings on ground level. Another aspect of this work is that a distinction is drawn between the oriental sore of the desert areas and that which is common in towns. The former is said to be a moist variety and it is of this that the gerbils and sousliks are the reservoirs. In towns oriental sore is of a dry type but so far evidence of a reservoir host has not been obtained. No doubt observers in other parts of the world where cutaneous leishmaniasis is common will attempt to discover if similar reservoirs exist in their localities. The whole of this work was published in Russian, with which few of us are



familiar. We are much indebted to Dr HOARE for his detailed review of this and other Russian work on leishmaniasis in the *Tropical Diseases Bulletin* for May last year.

#### HELMINTHIC INFECTIONS.

I think I have now come to the end of those tropical diseases in which important discoveries have been made during the war. It may be that in connection with some others there have been advances which should have been mentioned, but if so I am not familiar with them. There have been outbreaks of schistosomiasis amongst the troops and attempts have been made to find a better treatment for this helminthic infection than tartar emetic or fouth, but without any definite result as far as I am aware. Similarly there have been a number of cases of filariasis from the South-West Pacific area and a great deal of work to find a cure for this condition has been carried out in the United States. Though no specific remedy has been announced, a distinct advance has perhaps been made in the discovery that the cotton rat is liable to a filarial infection which may be conveniently used for testing chemotherapeutic agents. It may be that this animal, and also the dog by virtue of their filarial infection may be the means of discovering a long overdue remedy for filariasis.

We hear rumours that one or other of the sulphonamides, or penicillins, have a curative action in plague, cholera, relapsing fever, yaws and other tropical diseases, while quite recently it has been announced from France that workers in Madagascar have isolated from a plant growing there a glucoside named asiaticoside which they claim to be of value in the treatment of leprosy. It is clear that we need much more information before we can form any opinion as to the real value of these claims.

#### THE FUTURE OF TROPICAL MEDICINE.

From the review I have given I think it will be admitted that during the war considerable progress in our knowledge of tropical diseases has been made in several directions. The reason for this has been the great urgency for finding improved methods of protection and treatment for the very large number of non-immune soldiers it was necessary to send to tropical and sub-tropical countries. The rapidity with which supplies of remedies such as atabrine and sulphaguanidine were produced shows clearly what can be done when there is sufficient incentive. Now that the war is over I wonder whether research in tropical medicine will be carried on with the same enthusiasm and determination. In the United States interest in tropical medicine has developed considerably during the war and, with the very large number of men returning from overseas where they have been exposed to tropical infections, this interest will, of necessity be maintained. Schools of tropical medicine have been established and centres for the treatment and investigation of tropical diseases have been developed. It is safe to say that though before the war the U.S.A. was already in the forefront of tropical medical research in certain fields, it is

now advanced far beyond this, so much so that there is hardly a tropical disease that is not under investigation in some form or another in the United States at the present time. Many papers have been published and a number of books on tropical medicine and related subjects some of them very good indeed have appeared. Having found tropical medicine so interesting and productive it is hardly likely that workers in the U.S.A. will give up their enthusiasm for this branch of medicine—and no one would wish them to do so for they will undoubtedly continue to add much to our knowledge of this subject during the years that are before us.

It is perhaps unnecessary to refer in detail to the well-known facilities which exist for the study of tropical medicine in India. Courses of instruction are regularly conducted at the School of Tropical Medicine in Calcutta founded many years ago by Sir LEONARD ROGERS. Research is carried out there and at the Carmichael Hospital and many other centres where tropical diseases are treated. Research Institutes exist at Kasauli, Bombay, Madras and other places. Our knowledge of malaria is constantly being added to by the Malaria Institute of India. With its many highly trained and experienced investigators and its unrivalled opportunities for research, India will continue to make valuable contributions to our knowledge of tropical diseases. Other countries having tropical possessions, France, Holland and Belgium, were before the war in the forefront of tropical medical education and research and they have contrived to carry on to some extent. It is to be hoped that they will quickly recover from the setbacks they have had to endure. In South America investigations of the many diseases prevalent in that vast area have been carried on successfully for many years and have been productive of most important results. Tropical medical research has been going on for some years in South-East Russia and in China, and the war has not been able to bring it to a complete standstill. It will be evident that interest and developments in the field of tropical medicine will extend very widely now that the war is over.

### TROPICAL MEDICINE IN GREAT BRITAIN

Let us turn for a few minutes to the consideration of the position of tropical medicine in Great Britain, whose medical men surmounting many difficulties and enduring great hardships were in the early days pioneers in the study of tropical diseases in many lands. With the development of the British Empire, with its many interests in the tropics there was an increasing demand for medical men of tropical experience. Too often young doctors, but newly qualified, went out to practice medicine in the tropics where they had to learn, by painful experiences extending over years, what might have been taught them in as many months had facilities for such training existed. It was Sir PATRICK MANSON who, having suffered himself from this lack of teaching, first realized fully the urgent need for a centre in England where medical men going to the tropics could be taught something of tropical medicine and led to realize the great differences that exist between medicine as taught in the medical schools

at home and that which has to be practised abroad. The great difference, of course lies in the fact that so many tropical diseases are caused by animal parasites, so that when a patient comes under observation the first thought has to be—*is his condition the result of invasion of the body by one of these parasites which, if not recognized at once may determine a fatal issue before specific treatment can be adopted.* Sir PATRICK MANSON understood all this from long and painful experience in China, and it was his ambition to establish in London a school of tropical medicine where medical men going to the tropics for the first time would receive instruction which, though not making them experts, would enable them on arrival to deal intelligently with the patients who came under their care. Sir PATRICK MANSON knew that both laboratory instruction and clinical teaching should be carried on side by side and that with the one without the other there could never be a proper comprehension of what tropical medicine means. Accordingly with the help of JOSEPH CHAMBERLAIN and the Seamen's Hospital Society who had been convinced of the soundness of MANSON'S ideas, there was established in 1899 at the Royal Albert Dock the London School of Tropical Medicine, while in Liverpool, with the assistance of ALFRED JONES, the Liverpool School of Tropical Medicine came into being. Through these schools have passed many thousands of students who must have been eternally grateful for the instruction they had received when they found themselves in the unfamiliar surroundings of the tropics and confronted for the first time by tropical diseases of which they otherwise would have known nothing. The London School of Tropical Medicine at the Albert Dock, as many of you will remember comprised a Hospital for tropical diseases, Laboratories for teaching the laboratory side of tropical medicine, Research laboratories and an Out patient department. This was to my mind an ideal set-up which functioned very successfully for many years. In order to overcome what seemed to be the increasing difficulty of travel from London to the Albert Dock, the school, soon after the last war was moved to the old hotel building in Endelburgh Gardens, Euston, where the same departments, including the hospital were established. Apart from the convenience of being more accessible, the new Hospital for Tropical Diseases and School of Tropical Medicine offered no advantages over the old Hospital and School at the Albert Dock. Later as you know through a munificent gift from the Rockefeller Foundation there was established the London School of Hygiene and it was decided—in fact I am told it was a condition of the gift—that the School of Tropical Medicine should be taken away from the building at Endelburgh Gardens and amalgamated with the School of Hygiene under the joint title "London School of Hygiene and Tropical Medicine." This had the advantage of giving more space for the Hospital for Tropical Diseases, but it also appeared to me a retrograde step for now for the first time, the teaching of the laboratory side of tropical medicine was separated from the clinical side at the hospital. The close interdependence of the two was lost and gradually there arose the opinion which found expression in some quarters, that teaching

on the clinical side was not a necessity. This reveals a complete lack of understanding of the intimate association of the laboratory side with the clinical side which must exist if tropical diseases are to be understood and dealt with properly from the points of view of diagnosis, treatment and prevention.

While the changes which I have just outlined were taking place, a very significant development in the tropical medical world took place. I refer to the foundation at Hamburg of the Institut für Schiffs- und Tropenkrankheiten now I understand, raised to the ground. This had as its pattern the old London School of Tropical Medicine at the Albert Dock. There were similar hospital arrangements teaching laboratories and research departments all on a single site. Everything was on a larger scale than was the London School, and there can be no doubt that the Institute was well organized, possessed a highly competent staff and was very successful in every way. I paid several visits to the Institute in Hamburg and it was always with a feeling of envy or jealousy that I came away after having had to make some excuse for the absence of such a centre in London. To me it seemed inexplicable that it was not found possible for London—the centre of the British Empire, with all its Colonial possessions and Dependencies in tropical lands—to found a Tropical Medical Centre at least as good, if not better than the one in Hamburg.

#### THE URGENT NEED FOR A TROPICAL MEDICAL CENTRE IN LONDON

During the war through no fault of our own the facilities for dealing with tropical diseases and teaching tropical medicine have not improved. The Hospital for Tropical Diseases at Endleigh Gardens had to be evacuated for reasons of safety. It was subsequently seriously damaged by enemy action and was finally abandoned. That it was not past repair is proved by the fact that it was taken over by the Government and is now fully occupied by a department of the Ministry of Pensions. The London School of Hygiene and Tropical Medicine was also damaged on its eastern side but, in spite of this and the loss of staff on active service a number of short courses in tropical medicine were successfully and gallantly conducted for the benefit of medical officers of the allied nations who were going overseas. The present position in London seems to me deplorable. There is no Hospital for Tropical Diseases and the School of Tropical Medicine is housed in a building devoted chiefly to the teaching and study of hygiene and kindred subjects as applied to this country. There seems to be little prospect of the tropical medical section expanding. In fact, I understand that for teaching and research in hygiene and the establishment of new departments in this subject more space is urgently required. It does seem therefore that there is now an opportunity not only for formulating plans but for taking definite steps to establish a Tropical Medical Centre in London where sick people returning from the tropics could receive proper treatment either in hospital or as out patients where the teaching of tropical medicine in all its branches could be carried out in close association with a hospital for tropical diseases, and where research into the many problems still

awaiting solution could be carried on. I visualize such a Tropical Medical Centre as consisting of a Hospital of perhaps 300 beds where sick people from the tropics could be examined to determine in the first place whether they are suffering from some tropical disease or not and, if so, where they could have skilled and adequate treatment. An Out-patient Department which is properly advertised, so that casual and impecunious individuals from the tropics could obtain expert advice and treatment. Laboratories and Lecture theatres for the teaching of tropical medicine and Research Departments for research in the various branches of tropical medicine and allied subjects. The various sections should, ideally be in a number of separated buildings located close together on a single site or if this is found to be impossible in a central position in London, then in a single building of sufficient size to allow of expansion when this became necessary. In order to carry out such a plan satisfactorily it would probably be necessary to build, which is a great difficulty at the present time, though the provision of proper hospital facilities for the ever increasing number of people who will be returning to this country suffering from tropical diseases might be a sufficient excuse for breaking a rule at the present time. If such a scheme as that which I have tried to picture should be approved by those in a position to decide such matters it seems to me that a suitable site in a central position should be secured at once for it is certain that if this is not done no site will be available later on. Having secured such a site, a large notice should be erected announcing that this is the site for the New Hospital for Tropical Diseases and School of Tropical Medicine. If this were done the battle for a Tropical Medical Centre would be more than half won.

We have heard a great deal lately of Colonial developments and very large sums of money have been mentioned as earmarked for improvements in our Colonies not the least of which have been promises for improvements in the general health and living conditions of the natives. I can think of no single object better calculated to give such results in our tropical possessions than the establishment in London of a Tropical Medical Centre, where sick people from the tropics could be looked after and where medical men going abroad, and others coming here to study could be taught something about the modern methods of diagnosing and treating the diseases they would encounter where research would constantly be going on either at the Centre itself or in various parts of the world to which special teams of investigators had been sent. A great deal has been done recently to extend these facilities at the Liverpool School of Tropical Medicine which actually came into being a few months before the London School and it is now in a better position to undertake the treatment of patients and the teaching of tropical medicine. This is all to the good, but what is needed is a Tropical Medical Centre in London. It would restore the position formerly held by this country would be a monument to the many pioneers who devoted themselves to the study of tropical diseases and would be a centre of tropical medical activity worthy of the British Empire.

## COMMUNICATIONS

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### INVESTIGATION INTO THE EFFICACY OF SULPHADIAZINE IN THE TREATMENT OF MALARIA.

BY

R. V. COXON M.D. (LOND) M.R.C.P., CAPT R.A.M.C.\*

*Graded Physician*

AND

WILLIAM HAYES M.B.C. (DUBL.) F.R.C.P.I. D.P.H., MAJOR, R.A.M.C.,

*Specialist in Pathology*

*From a Military Hospital India Command*

and

*The Central Military Pathological Laboratory of India*

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The present investigation arose out of a report by COGGESHALL, MAIER and BEST (1941) from the Canal Zone in America, where thirteen cases of malaria were treated by sulphadiazine with apparently successful results in ten of them. These experiments, however did not seem to have been sufficiently well controlled either clinically or by laboratory examinations to warrant any definite statement as to the efficacy of the drug.

#### SCHEME OF INVESTIGATION AND METHODS EMPLOYED

(1) *Number of cases*—Twenty cases, including both fresh and relapsing cases of benign tertian (B T) fresh and relapsing cases of malignant tertian (M T) and one case of quartan malaria, were treated with sulphadiazine alone. A number of other cases also were treated with sulphadiazine but in these the treatment was supplemented with some other form of therapy such as

\*Our thanks are due to Col. J. D. S. CAMERON late R.A.M.C., at whose instigation this enquiry was undertaken to Lt.-Col. A. W. D. LEIDHMAN and Lt.-Col. R. N. PHRAKE, for much kindly help and encouragement throughout the investigation to Major MARY McHUGH, I.A.M.C. for carrying out the laboratory work during the latter part of the research to Capt. P. C. WINGATE, R.A.M.C., for painstaking assistance with the follow-up and also to Col. G. MOULSON and Lt.-Col. PHRAKE for permission to publish this paper.

plasmaquine, quinine or atabrin. These cases are not included in our series, but a few points of interest in connection with some of them will be mentioned in the course of this paper.

(2) *Control cases*.—The variability in the natural history of an attack of malaria makes the accurate assessment of the results of treatment very difficult unless an adequate series of control cases is used for comparison. These controls are of two types—firstly those undergoing no form of treatment apart from the purely symptomatic variety such as the administration of aspirin and, secondly from the point of view of the study of the incidence of relapse, those treated with quinine, atabrin and plasmaquine in accordance with routine Army practice.

Our series included ten untreated controls. In practice it was found that by the time a patient had reached hospital and the diagnosis had been established, his attack had usually been proceeding for some 3 days so that treatment could not be instituted until the 4th day of the disease. Since we found that the day on which the average treated case became parasite-free was the 12th day of the disease, although only the 8th day of treatment—in providing controls for these cases it was necessary to allow a period of observation of at least 12 days. Accordingly in our untreated controls the attack was permitted to take its course with only non specific, symptomatic treatment for 12 days or in some cases, rather longer. Results of quinine-atabrin-plasmaquine treatment were assessed from the effects of this form of treatment on the untreated controls at the conclusion of their experimental period, on cases admitted and treated normally and from reports from extraneous sources.

(3) *Assessment of effect of treatment*.—This was done, in the first place, by clinical observation of the cases, the most important criterion being the cessation of pyrexia. Secondly progress was followed by serial laboratory examinations of the blood carried out at definite intervals during treatment. These examinations included both the performance of parasite counts and the observation of the forms of the parasite present.

(i) *Parasite Counts*.—Whenever possible, samples of blood were taken in the morning between the hours of 9 and 10.

Four c.c. of blood were withdrawn by venepuncture and discharged into a small corked tube containing an optimal amount of solid ammonium and potassium oxalate as anticoagulant to ensure isotonicity as recommended by WINTROBE. These samples were received at the laboratory usually within 1 hour of taking and all laboratory examinations carried out on them within 2 hours of receipt.

If positive counts are to be accurate and if negative findings are to be of any value—an important factor in an investigation such as this—a relatively large volume of blood must be examined. This necessitates the use of a thick film method. Moreover an absolute method of counting is desirable since the enumeration of parasites relative to some other variable such as red or white blood cells which may vary not only in number (thus introducing an additional and tedious estimation for every count performed) but also in their distribution

in the film (a factor which cannot be accurately assessed) must increase the experimental error. The method used was a modification of that of PRESCOTT and BREED for the enumeration of the total bacteria in milk and is similar in principle to CHRISTOPHERS' method for the counting of malaria parasites.

The oxalated blood to be counted is drawn up to the 20 c.mm. mark into a Sahli haemoglobinometer pipette. The end of the pipette is wiped and the blood is carefully discharged on to a clean, grease-free slide and spread accurately and evenly over an area of 2 sq. cm. The film is allowed to dry at room temperature on a level surface. It is then dehaemoglobinized with distilled water for 30 seconds, stained by Field's stain and left standing in the vertical position to dry. Field's stain is easy and quick to use, the staining of each slide taking only about 10 seconds. Interpretation of the films is easy once a little experience has been acquired.

In order to perform a count, the diameter of the microscope field using the 1/12 inch objective, is measured and from this the area of the field is calculated. If we know the average number of parasites per field it is easy to calculate the number present in the 2 sq. cm. of film. But this is the number in 20 c.mm. blood since this was the amount of blood used in making the film. The number of parasites in 1 c.mm. blood is, therefore, known and all results were thus expressed. In counting the edges and central parts of the film were given equal consideration. The number of fields counted in order to obtain an average depends on the concentration of the parasites and the evenness of their distribution.

Before a sample of blood was reported negative at least 100 fields representing 0.5 c.mm. blood, were examined.

The method gave good and consistent results in practice was quick and simple to perform and required no special apparatus.

The advantage of the thick film method over the thin in assessing the effects of treatment is shown by comparing the average day on which the blood first failed to show parasites as judged by both methods for the first twelve cases under treatment with sulphadiazine. This figure was 3.7 by the thin and approximately 8 days by the thick film method. On the average the thin film failed to reveal parasites after a reasonably thorough search when their number had dropped to less than 100 per c.mm. This counting range, lying between the limits of the two methods, is the critical one for investigation when the effects of a new drug on the parasite are being examined.

(ii) Observation of the forms of the parasite present was made on thin films stained by Leishman's stain. Lack of time precluded differential counts except in the case of MT gametocytes and the predominant form present, whether early or late trophozoite or schizont, only was noted.

It was considered that one of the chief factors which would determine the usefulness of any new anti-malarial drug and one which should be given a prominent place in the assessment of effects was the ability of that drug to effect a cure and not merely a suppression of the infection. This property of the drug was compared with that of the standard Army treatment by following up the cases after their discharge from hospital. The likelihood of fresh infections arising to complicate the position was reduced to a minimum by transferring the patients on discharge to a Convalescent Depot situated in an area where the altitude precludes the possibility of fresh infection. We were thus able



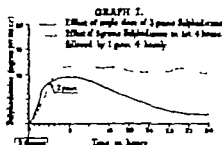
to form a rough idea of the relapse rate of cases treated by sulphadiazine on the one hand and by the quinine stebrin plasmoquine course on the other

(4) *Dosage of the drug*—Routine estimations of the blood sulphadiazine level were made at definite intervals throughout treatment in all cases.

*Method*—The basis of the method used was that of WIGGERS (1939) and depends on the development of a yellow colour of an intensity proportional to the concentration of the drug when compounds of the sulphonamide group are mixed with a solution of p-dimethyl-amido-benzaldehyde. 1.0 c.c. oxalated whole blood was added to a mixture of 20 per cent. trichloroacetic acid (0.8 c.c.) and HCl NaCl buffer (1.2 c.c.). The proteins precipitated at once and were removed by centrifuging. 1.0 c.c. of the clear supernatant fluid was transferred to a narrow tube and 0.2 c.c. of a 1 per cent. solution of p-dimethyl-amido-benzaldehyde in acid-alcohol added. The resultant yellow colour was matched against a series of potassium chromate-dichromate standards which had previously been standardized against known, weighed concentrations of sulphadiazine treated in precisely the same manner as the blood.

The method estimates only the free drug and not the acetylated form.

The dosage of sulphadiazine employed in the majority of cases was that generally recommended for the treatment of pneumonia by the same drug (FINLAND *et al.*, 1941), that is, a loading dose of 5 grammes in the first 4 hours followed by 1 gramme 4-hourly. Some initial experiments were carried out, however, to determine (a) the rate of absorption and excretion of a single, initial dose of 3 grammes, and (b) the effect of an initial dose of 5 grammes in the first 4 hours followed by 1 gramme 4-hourly in maintaining the blood level. The results of these investigations are shown in Graph I



Using the latter dosage we found that, on the average, the concentration in the blood measured 24 hours after the first dose was about 10 mg per 100 c.c. and tended to remain at approximately that level throughout the course. The slow rate of fall after the initial dose alone suggested that both economy in the use of the drug and the maintenance of a satisfactory blood level might be effected by lowering the maintaining dose to 1 gramme 6-hourly. Later experiments showed that patients on this dosage yielded an average blood level of 9 mg per 100 c.c.

The effects of varying dosage will be dealt with later

(5) *Erythrocyte Sedimentation Rate*—The reported statement of certain workers that a normal E.S.R. is of value as a criterion of cure as opposed to

a suppression of infection after treatment in malaria suggested that an examination of this claim might usefully be carried out as an investigation subsidiary to the main point at issue.

True E.S.R. values only were used. These were obtained by correcting all results for anaemia by means of the Corpuscular Volume.

(6) *Examination of Sternal Punctures*—We had thought it desirable that all patients should have their peripheral blood examined after an injection of adrenaline and their sternal bone marrow searched for parasites at the conclusion of their course of treatment before being pronounced parasite free. These examinations were carried out on the first three patients but unfortunately owing to exigencies of service it was found impossible to continue them.

### EFFECTS OF SULPHADIAZINE TREATMENT

#### (1) *Effects on Pyrexia*

It was found that, on the average in the B.T. cases fever had subsided after 4½ days of treatment during which time an average total dosage of 28 grammes of sulphadiazine had been administered. The M.T. cases became afebrile after an average period of 6 days while the quartan case had his last rigor on the 4th day after treatment had been started. Thus, judging only by clinical appearances it seemed that the B.T. cases had recovered after 4 to 5 days and the M.T. patients after 6 days of treatment.

On the average in these cases treatment was not begun until the 4th day of the disease.

Of the ten untreated controls four had spontaneously lost their fever within 12 days of onset—one on the 2nd day, one on the 3rd and two on the 6th day. In the remaining six cases the fever was virtually unaltered in degree at the conclusion of their 12th day experimental period. Four of these latter remained pyrexial until the 17th day when their fever was terminated by the administration of quinine. It is worth noting that the cases which became afebrile spontaneously were all cases of relapsing B.T. malaria.

#### (2) *Effects on Parasite Counts*

The average time required for the blood to become parasite free after the initiation of treatment was 8 days for both B.T. and M.T. cases. The shortest time required for sterilization was 4 days and the longest 11 days, which applied to only one case. This refers only to the asexual forms of the parasite and not to gametocytes which will be dealt with later.

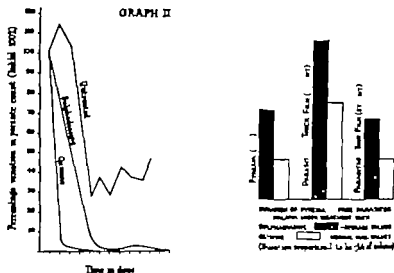
It has already been pointed out that, for the first twelve cases under treatment, thin films became negative on the average in 3.7 days.

Of the ten untreated controls not one achieved a negative blood examination within the 12 day period of observation.

On the average in the sulphadiazine treated cases the count began to show

a significant fall shortly after the beginning of treatment and thereafter in all cases with one exception, declined steadily to zero. The parasite counts in the untreated controls, on the other hand, tended to pursue an irregular course until the disease was controlled by quinine. Six of these cases, after a preliminary fall maintained a fairly steady count throughout their course. Two cases showed a progressive rise followed by a gradual decline until given quinine, while in another the count rose steadily from a low value until the 12th day when quinine was given. Only one case showed a steady and progressive fall from an initial count of 3 600 parasites per c.mm. to 14 per c.mm. on the 13th day when, unfortunately quinine was administered.

Only one sulphadiazine treated case—the exception mentioned above—was encountered where it appeared possible that the parasites may have been proving resistant to the drug. In this patient the count had remained constant at between 3,500 and 4 000 per c.mm. for 10 days in a blood drug concentration of between 9 and 10 mg per 100 c.c. The drug dosage was increased but the count was still 1,500 parasites per c.mm. with a blood drug level of 18 mg. per 100 c.c. on the 14th day. As ill-luck would have it, this patient was inadvertently given quinine at the critical juncture by an over zealous nursing orderly so that no definite conclusions can be drawn from his behaviour. For this reason the case has not been included in our series.



Quinine usually produces a rapid fall in the pyrexia as well as in the parasite count to a very low level in 48 hours or less and complete clearing of the blood of parasites within 5 days as compared with the average of 8 days required by sulphadiazine.

The effects of sulphadiazine as compared with those of quinine on the circulating parasites are summarized in Graph II and the appended diagram.

The graph shows the curve of fall of the parasite count following sulphadiazine the fall being indicated by the average percentage decrease in the number of parasites from their initial value at the beginning of treatment for the first twelve cases. The curve is compared with that usually found in quinine treated cases and also with the curve produced by averaging the counts given by our ten untreated controls. This latter curve should not be regarded as anything more than a rough approximation to the truth since these cases not only displayed wide variation in their behaviour but parasite counts were not performed on them with the same regularity as on the treated ones. The curve does however convey some impression of the progress of the untreated cases and enables the other two to be seen in clearer perspective.

### (3) *Effects on the Asexual and Sexual stages of the Parasite*

It was hoped to gain some information on the mode of action of the drug by observing whether or not the development of any stage of the life cycle of the parasite in man was inhibited in those patients under treatment.

(a) *Asexual stages* We have mentioned that, shortly after the beginning of treatment, the parasite count began to fall in the typical case and, thereafter continued to fall steadily to zero. Throughout this period the regular asexual cycle was maintained and was in no way different from that observed in the same case before the fall occurred or in the untreated controls. Any inhibitory or lethal action of the drug might, therefore be due to action on either any one or all of the stages of the cycle. It is to be regretted that stress of work has so far prevented any investigation into the effects of the drug on malaria parasites *in vitro*.

(b) *Sexual stages* The identification and counting of gametocytes of *P. vivax* in the thick film is a rather difficult and time-consuming task. Our observations on the sexual stages were therefore, restricted to the gametocytes of *P. falciparum* which presented no difficulty in these respects. Three cases of fresh and two cases of relapsing M.T. malaria were treated with sulphadiazine. The gametocytes appeared to be unaffected by the drug appearing at about the expected time and undergoing the normally expected rise and fall in one case reaching a maximum count of 3 000 per c.mm. during the course of treatment. In no case did gametocytes fail to appear. Gametocytes were also noted in the blood of the majority of B.T. cases both fresh and relapsing and in the quartan case during the administration of sulphadiazine.

### (4) *Effects of Dosage other than Standard*

In view of the fact that sulphadiazine appeared to be tolerated well by our patients it was decided to try the effect of a dosage higher than standard. Several patients were given a maintenance dose of 2 grammes 4 hourly instead of the usual one. In these cases blood drug levels of from 17 to 20 mg per 100 c.c. were recorded. Incidentally 20 mg per 100 c.c. is generally regarded

as the ceiling value which it is inadvisable to exceed. We found that the response was not materially different from that produced by the standard dosage. An attempt was also made to reduce the dosage and a further group of patients received 2 grammes 4 hourly for the first 24 hours followed by 1 gramme 6-hourly. These patients maintained a blood concentration of 9 mg per 100 c.c. and again the response was the same as that produced by the standard dosage except in the case of one man whose blood level reached only 7.5 mg per 100 c.c. This man subsequently responded rapidly when his blood level was raised to 15 mg per 100 c.c. by increased dosage. It is possible that a concentration of between 8 and 9 mg per 100 c.c. is close to the critical lower level necessary for effectiveness.

### (5) *The Relapse Rate*

A few of our cases under treatment with sulphadiazine relapsed while still in hospital, often within a few days of the cessation of treatment. These cases responded to a further course of the drug. A few were terminated by quinine and these are not included in our series. Of the series of twenty cases treated with sulphadiazine and sent to the Convalescent Depot for observation, fifteen—i.e. 75 per cent.—relapsed within a period of 1 month from the cessation of treatment. The type of malaria with which these patients were infected and the number of each type which relapsed are shown in the accompanying table.

RELAPSE RATE AMONG DIFFERENT TYPES OF MALARIA  
TREATED WITH SULPHADIAZINE.

Type of Case	Number of Cases.	Number which Relapsed.
B.T. (R)	12	9
B.T. (F)	5	5
M.T. (R)	1	1
M.T. (F)	1	0
Quartan	1	0
TOTAL	20	15

This high relapse rate compares unfavourably with that in a group of fifteen similar cases who were treated by the standard Army method of 4 grammes of quinine, 1.5 grammes of atabrin and 0.15 gramme of plasmoquine given over a period of 14 days. These fifteen cases showed only one relapse after a period of 1 month. The case previously mentioned who appeared to be resistant to sulphadiazine and whose disease was inadvertently terminated with quinine also received the standard Army treatment and relapsed within the month. He has not been included in the series of quinine treated controls, however, since he had previously had an intensive course of another drug.

### (6) *Erythrocyte Sedimentation Rate in relation to the Relapse Rate*

No correlation was found to exist between the E.S.R. and either the severity of the attack or the presence or absence of relapse over the time of observation. Of the sixteen cases which relapsed, the E.S.R. was normal in nine, doubtful—i.e. above 5 and below 8 mm. drop in 1 hour—in three, and was definitely raised in only four at the time of discharge from hospital. On the other hand of fourteen cases which had not relapsed after an average period of 39 days from the time of discharge, the E.S.R. was normal in eight, doubtful in two and in four was definitely raised on leaving hospital. These latter results, of course, have not the significance of our findings in the relapsed cases since the four cases with the increased E.S.R. may have relapsed at a later date.

It must be emphasized that all E.S.R. readings were corrected for anaemia.

### COMPLICATIONS OF TREATMENT

Most patients took the drug without any gastro-intestinal disturbance. A few vomited at the outset, possibly owing largely to their fever but even these, with the help of an alkaline mixture absorbed sufficient to maintain a satisfactory blood concentration. It has been mentioned that the usual concentration maintained was about 10 mg per 100 c.c. and also that some patients reached a level of 20 mg per 100 c.c. on a higher dosage. The only complications met with, apart from the comparatively unimportant occurrence of vomiting in a few patients, were renal. Renal colic affected four patients. In two of them haematuria also occurred but no crystals of the drug were identified in the urine. It is noteworthy that only one case was affected in the month of February when the weather was cool, but that three occurred in rapid succession later on when the weather became hotter.

In view of the well known capacity of the sulphonamide group of drugs for producing leucopenia, white blood cell counts were performed on all cases at regular intervals throughout treatment. Malaria itself of course causes leucopenia but at no time during the investigation was a dangerously low white cell count referable to sulphadiazine therapy observed.

### DISCUSSION AND SUMMARY

Our primary object was to discover whether sulphadiazine was capable of replacing quinine or the other drugs commonly used in the treatment of malaria in view of a possible future shortage of quinine and of the fact that sulphonamides are relatively simple chemicals to manufacture. Our aim was to ascertain whether the new drug would be effective in relapses the occurrence of which is the chief imperfection of existing methods of malarial therapy. In fact, the malaria problem today is largely that of the relapsed case.

Lastly and quite incidentally we wished to find out the value, if any, of the erythrocyte sedimentation rate as an indicator of the likelihood of relapse.

As has been mentioned, the variability in the natural history of an attack of malaria renders the evaluation of treatment a somewhat complicated problem, especially when the enquiry is limited to a comparatively small number of cases. Nevertheless, the following facts emerged quite clearly from the investigations described: firstly that, whereas the cases treated with sulphadiazine had their blood cleared of parasites after an average period of 12 days from the onset of their attack and of 8 days from the initiation of treatment, all of the ten untreated controls had parasites circulating after the same lapse of time. The latest day on which a treated case became parasite-free was the 17th day of disease and the 12th day of treatment in one case. Secondly while all the treated cases had parasite free films after 17 days the control cases who were allowed to go untreated for the same period all had parasites still present at the end of it. Thirdly all the treated cases, with the single exception of one very heavily infected M.T. case who did settle down later, were afebrile by the 13th day of attack while six of the ten controls continued to run a high temperature after the same interval.

It seems reasonable to conclude therefore that sulphadiazine does exert an anti malarial effect but that its effect on the temperature and in clearing the peripheral blood of parasites is slower than that of quinine or stebrom. In addition, it may be remarked that these findings illustrate two points of paramount importance in an investigation such as this: firstly the inestimable value of controls and, secondly the indispensable part played by laboratory co-operation and the use of the thick film method of examination for parasites in the management of malaria.

Investigation into the relapse rate following sulphadiazine treatment, limited though it was both in respect of length of time of observation and of the number of cases observed, made it clear that the drug is inefficient as a para-tertobol agent and that treatment by it carries a much higher relapse rate than does combined treatment by quinine, stebrom and plasmoquine. This fact alone rules out its usefulness in the routine treatment of malaria. In addition to this there is the tendency of the drug to produce renal complications, constituting a serious disadvantage in an anti malarial remedy which, from its action, finds its maximum use under tropical conditions where it is difficult to prevent concentration of the urine.

Finally the failure of the erythrocyte sedimentation rate as an indicator of the probability of relapse renders it worthless as a guide in the management of malaria.

It is already known that a small dose of certain members of the sulphonamide series of drugs now in general use will cure the invariably fatal *P. knowlesi* infection of monkeys. The fact that that member of the series known as

sulphadiazine exerts a definite anti-malarial effect in the principal types of plasmodial infection of man raises the hope that further chemical research may bring to light a related compound which will prove to be more useful and may help to reduce the liability to relapse which is the main failing of existing methods of malarial therapy

### CONCLUSIONS

- 1 Sulphadiazine has a definite anti malarial effect on the asexual stages of the parasite in man.
- 2 Its action is inferior to and slower than that of quinine and atebirin in controlling an attack of malaria.
- 3 Treatment with sulphadiazine carries a much higher relapse rate than does combined treatment with quinine atebirin and plasmoquine.
- 4 Sulphadiazine is inert against the gametocytes of *P. falciparum* and probably also against those of *P. vivax*.
- 5 The corrected erythrocyte sedimentation rate (E.S.R.) is of no value in assessing the probability of relapse after treatment in malaria.

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## ANAEMIA ASSOCIATED WITH THE SICKLE CELL TRAIT IN BRITISH WEST AFRICAN NATIVES

BY

R. WINSTON EVANS MAJOR, R.A.M.C.,\*  
*Pathologist, West African Military Hospital.*

The term sicklaemia or sickle cell trait, is applied to those persons whose sealed blood cells exhibit sickling on standing from several to 36 hours, but who show no evidence of a haemolytic anaemia. Latent sickle cell anaemia is a condition occurring in sicklaemic persons who present indefinite blood changes and few haematological signs and symptoms but who give a history suggestive of attacks of acute haemolytic crises. Conditions converting the sickle cell trait into the active anaemia are said to be local or general anoxaemia, acute toxic conditions, trauma and other factors causing blood loss and slowing of the blood stream. WHITBY and BRITTON (1942) list the following symptoms as likely to occur in sickle cell anaemia: weakness, dyspnoea, dizziness, vomiting, epigastric pain, splenic pain, symptoms of heart disease and pain in joints and muscles. Various authors describe the following signs as commonly occurring: ulceration of legs, general enlargement of lymph glands, yellow sclerae or conjunctival jaundice, enlargement of liver and spleen, oedema of legs, low blood pressure, cardiac hypertrophy and cerebral necrosis. In a population a large percentage of which is afflicted with yaws, helminthic infestations, chronic malaria, blackwater fever, dysentery and dietary deficiency diseases, it is by no means always easy to evaluate symptoms, and it is wise to exercise caution in attributing to active sicklaemia a legion of signs and symptoms that may be found in a patient who exhibits sicklaemia with an anaemia. It is considered that the only signs and symptoms that should be definitely attributed to active sicklaemia are those arising as a result of the thrombotic haemorrhage and necrosis which are features of the disease. Such signs and symptoms may naturally involve any organ, but in my experience none was affected with sufficient regularity to produce a characteristic picture.

\* I am very much indebted to Lt. Col. J. C. LEEDHAM-GREEN, F.R.C.S., R.A.M.C., O.C. Surgical Division for his surgical opinion and help in the preparation of this paper and especially for performing the splenectomy in Case 4.

My thanks are due to Lt.-Col. W. M. MACNAUGHT, R.A.M.C., and Major R. M. MURRAY LYON, R.A.M.C., for allowing access to patients and notes of the Medical Division.

I am grateful to Brigadier R. A. HIPPLE, O.B.E., M.C., D.D.M.A., West Africa, and Brigadier G. M. FINDLAY, C.B.E., for their interest in this communication.

The problem of diagnosis of sickle cell anaemia is further complicated by the high incidence of the sickle cell trait among West African natives. In a previous communication (EVANS 1944) it was pointed out that the trait could be demonstrated in 15.5 per cent. of fit male natives and in 25 per cent. of men admitted to hospital suffering from various acute and chronic diseases. In this paper eight cases of anaemia associated with sicklaemia are described to illustrate the difficulties that may occur in the diagnosis of sickle cell anaemia, and the importance of other diseases in precipitating attacks of sickle cell haemolytic crises. The case reports have been grouped under two headings—

*Group I* Anaemias in which a cause other than sicklaemia could be demonstrated.

*Group II* Sicklaemia in patients suffering from acute toxic conditions.

I have given a separate commentary on the case or cases occurring in each group.

## GROUP I

### A. MEGALOBLASTIC (NUTRITIONAL TYPE) ANAEMIA IN PATIENTS SHOWING THE SICKLE CELL TRAIT

#### Case 1

Pte. S. M., a Gambian, aged 15 years, was admitted to hospital on 11.4.42 with a simple fracture of the fifth metatarsal bone, due to a slight injury 48 hours previously when an empty petrol tin had fallen on his foot. He was thin and of poor physique but gave no history of previous illness.

Temperature 103° F., pulse 100. Oedema of dorsum of foot and tenderness over the fifth metatarsal. X-ray confirmed fracture in good position and showed no evidence of bone disease. During the next few days the oedema increased and an abscess developed with necrosis of the underlying skin. An incision was made and the necrotic skin excised, revealing a greyish necrotic floor and foul-smelling thin and sanguinous discharge. For 9 days after admission his general condition deteriorated. His temperature fluctuated between 100 and 105° F. His conjunctivae were jaundiced and his spleen enlarged. His urine was dark in colour and showed a cloud of albumin. At this time his blood picture was as follows—

*Peripheral Blood.*—R.B.C., 1,240,000 cells per c.mm. haemoglobin, 30 per cent.<sup>+</sup> W.B.C. 38,000 cells per c.mm. colour index, 1.4 polymorphonuclears, 54.4 per cent. lymphocytes, 24.6 per cent. monocytes, 5.6 per cent. eosinophils, 6.4 per cent. myelocytes, 3.2 per cent. metamyelocytes, 5.2 per cent. plasma cells, 0.4 per cent. Red cells showed marked anisocytosis with megalocytes.

Sickling was demonstrated *in vitro* and *in vivo*. Malaria parasites were not found in thick films.

*Sternal Bone Marrow.*—Polymorphonuclears, 28.6 per cent. lymphocytes, 0.8 per cent. monocytes, 0.8 per cent. eosinophils, 1.2 per cent. myeloblasts, 1.0 per cent. myelocytes, 6.0 per cent. metamyelocytes, 10 per cent. plasma cells, 0.8 per cent. proerythroblasts, 10.4 per cent. megaloblasts type A, 21.3 per cent., megaloblasts type B, 7.2 per cent. megaloblasts type C, 3.4 per cent. normoblasts all types, 10 per cent. Megakaryocytes present. Ratio of myeloid cells to nucleated red cells, 3:2. Marrow intensely hyperplastic and megaloblasts replaced the usual series of normoblasts.

A diagnosis of megaloblastic anaemia (primary dietary deficiency type) with sicklaemia was made and the patient was put on to liver extract and marmite by mouth.

All haemoglobin percentages are worked out on the basis that 100 per cent. Hb equals 14.5 grammes Hb per cent.

After 9 days of this treatment his general condition had improved and he was no longer complaining of pain in his foot. His spleen was no longer palpable. After another 10 days the ulcer showed healthy granulation tissue and was healing in at the edges. At this time his blood picture was as follows:—

*Peripheral Blood.*—R.B.C., 3 190 000 cells per c.mm. haemoglobin, 63 per cent. W.B.C., 7 000 cells per c.mm. colour index, 1.0

*Sternal Bone Marrow.*—The intense hyperplasia had disappeared and a normal series of normoblastic developmental forms had replaced the megaloblastic series.

For the next 14 days the patient a dose of liver and marmite were inadvertently stopped, and at the end of this time his general condition had deteriorated as had also the ulcer which had lost its former healthy appearance, discharged a thin watery exudate and showed no further sign of healing. On resumption of liver extract and marmite he again improved rapidly and 10 days later was discharged from hospital when his peripheral blood count was R.B.C. 4 400 000 per c.mm. haemoglobin 81 per cent. W.B.C., 8 000 per c.mm. colour index, 0.9

Six months after discharge he was still well and serving as a soldier

### Case 2.

Pte. J. B., a Gambian aged 32 years, was sent to hospital on 3.9.42 as a case of acute osteomyelitis of the right tibia of 2 days' duration without any history of injury. He complained of pain and was acutely tender over the middle third of the shaft of the right tibia. There was some local oedema. His temperature was 103 F. pulse 115. Skin hot and dry. conjunctivae slightly icteric. no palpable enlargement of liver or spleen. A diagnosis of acute osteomyelitis was considered to be unlikely as in spite of the exquisite local tenderness the patient allowed passive movements of all the joints of the affected limb and percussion of the heel against the tibia without expressing the least resentment. Further investigations were then carried out. Urine, clear dark amber colour. albumin a trace. no bile, no cells, no casts, no *Leptospira icterohaemorrhagiae* on dark ground illumination of sediment. Blood films negative for malarial parasites. Cerebrospinal fluid clear and colourless without clot, containing one lymphocyte per c.mm. globulin negative, culture sterile. Blood culture sterile after 72 hours incubation.

*Peripheral Blood.*—R.B.C., 3 800 000 per c.mm. haemoglobin, 75 per cent. W.B.C., 20 000 per c.mm. colour index 0.98 polymorphonuclears, 79.5 per cent. lymphocytes, 11.0 per cent. monocytes 8.0 per cent. myelocytes, 0.5 per cent. metamyelocytes, 1.0 per cent.

Red cells were shown to sickle *in vitro*

Three days later the jaundice of the conjunctivae was more pronounced and a second blood count showed R.B.C. 3,200 000 per c.mm. haemoglobin, 61 per cent. W.B.C., 17 000 per c.mm. colour index, 0.88.

*Faeces.*—Normal in colour and repeated examination for helminth ova were negative.

*Gastric Juice.*—Fasting juice showed no free HCl and no organic acid. Juice after alcohol showed no free acid.

His sternal bone marrow showed much hyperplasia with 10 per cent. megaloblasts, and a diagnosis of megaloblastic anaemia with sickle anaemia was made. Liver extract and marmite were exhibited by mouth and his steady improvement on this treatment was reflected in successive blood counts. On discharge from hospital on 20.10.42 his peripheral blood count was R.B.C., 4,850 000 per c.mm. haemoglobin, 96 per cent. W.B.C. 8 000 per c.mm.

### Commentary

The importance of these two cases lies in the fact that but for sternal bone marrow examination the underlying megaloblastic anaemia would not have been discovered and they would have been diagnosed as classical sickle cell anaemias. For apart from the sickling of the red cells, they presented those signs and symptoms which are described as occurring in that disease, *viz.* leg ulcer bone pains, fever jaundice, splenomegaly, leucocytosis and dark

coloured urine. These signs and symptoms may of course have been due to sickling *in vivo* caused by the anoxia produced primarily by the megaloblastic anaemia. The success claimed for liver therapy in some cases of sickle cell anaemia may well be due to failure to distinguish between sickle cell anaemia and a megaloblastic anaemia associated with sicklaemia. Although true megaloblasts have been reported to occur in sickle cell anaemia (DIGGS and BEN, 1939) I prefer to regard these cases in which such cells are found as instances of a liver principle defective type of anaemia.

For the purposes of clarity it is pointed out that the cells here described as megaloblasts are cells with an open reticular nuclear structure, occurring in the bone marrow only in pernicious and other allied liver principle defective types of anaemia. The large early haemoglobinized cell with a well patterned coarse nuclear structure often seen in haemolytic anaemias and in conditions of prolonged blood loss is regarded as a modified normoblast. The classification adopted is that of ISRAELS (1941).

No Kahn test was done in either of these patients but in the light of subsequent experience I am of the opinion that a concomitant yaws infection was responsible for the two features of leg ulceration and bone pains. Although I was working in a community among whom ulceration of the legs was common, in almost every case where ulceration associated with the sickle cell trait was investigated, yaws proved to be the aetiological factor and no greater incidence of leg ulceration was found among those who showed the sickle trait.

## B HYPOPLASTIC ANAEMIA ASSOCIATED WITH THE SICKLE CELL TRAIT

### Case 3

Pte Cham, a Gambian aged 24 years, reported for dental treatment on 13.3.42. On examination he was found to have ulcerative gingivitis associated with swelling of the lips round the angle of the jaw. The ulceration responded to treatment (chromic acid and hydrogen peroxide) and he was discharged on 18.5.42. A week later he returned to hospital and on re-examination the original ulcer was found to be in approximately the same position as when he first reported for treatment. He was again treated with chromic acid and hydrogen peroxide. The ulceration spread rapidly despite treatment resulting finally in complete transection.

He was admitted to hospital as an in-patient on 25.5.42 owing to the noticeable degree of anaemia. There was tenderness over the ribs and sternum on percussion and the conjunctivae were jaundiced, otherwise physical examination was negative. The progress of the case was one of steady deterioration with one short remission following a blood transfusion.

### Investigations

*Blood Count.*—5.6.42 R.B.C. 1,340,000 per c.mm. haemoglobin, 35 per cent. W.B.C. 2,000 per c.mm. colour index 1.1 polymorphonuclears, 18.5 per cent. lymphocytes, 64.5 per cent. monocytes, 13.5 per cent. eosinophils, 3.0 per cent. basophils, 0.5 per cent.

Sickling of the red cells was demonstrated *in vitro*.

*Blood Count.*—9.6.42 R.B.C., 1,580,000 per c.mm. haemoglobin, 30 per cent. W.B.C. 800 per c.mm. colour index 1.0 Polymorpha, 8.0 per cent. lymphocytes 86.5 per cent. monocytes, 3.5 per cent. eosinophils, 1.5 per cent. plasma cells, 0.5 per cent.

*Sternal Bone Marrow*—28.42 Polymorphonuclears, 4 per cent lymphocytes, 26.5 per cent. monocytes, 2.5 per cent eosinophils 1.25 per cent. myeloblasts, nil myelocytes, 1.5 per cent. metamyelocytes, 0.75 per cent. proerythroblasts, 1.25 per cent. normoblasts type A, 8.0 per cent. normoblasts type B 26.0 per cent. normoblasts type C, 20.75 per cent. megakaryoblasts nil.

Total nucleated cell count was only 13 000 per c.mm.

*X-ray Chest*—Lung fields clear

*Faeces*—No amoebae and no helminth ova.

*Blood Smears*—No protozoal or helminth parasites.

*Summary of Autopsy Findings*—Marked emaciation. Conjunctivae jaundiced. Necrotic ulcerative lesions of mouth upper and lower gums, fauces and palate. Scattered petechial haemorrhages. Liver normal in size on section pale with focal necrotic changes. Spleen normal in size on section pale with much increase in fibrous tissue. Lungs hypostatic congestion. Brain pale and showing early cerebral softening. Other organs nil abnormal demonstrated.

### Commentary

This case is of interest on account of the hypoplastic condition of the bone marrow as revealed by sternal puncture, and the failure to respond to treatment. It was not possible to investigate any causative factors in the production of the bone marrow hypoplasia certainly none of the usual potentially toxic drugs, benzol, radio-active substances, arsenic sulphapyridine etc. had been exhibited to my knowledge.

Although sickling of the red cells was demonstrated *in vitro* and *in vivo* this was not considered to be a case of sickle cell anaemia, but an instance of hypoplastic anaemia associated with sicklaemia. It may of course be argued that this is an example of sickle cell anaemia terminating in an aplastic phase. Apart from the necrotic lesions due to the bone marrow aplasia, the thrombotic tendency of the sickle cells formed as the result of the anoxia contributed largely to the widespread capillary thrombosis and associated necrosis of the cerebrum and other organs.

## C ANAEMIA WITH SICKLAEMIA IN HOOKWORM INFESTATION

### Case 4

Pte. Ch. about 32 years old, a native of the Cameroons, was admitted on 28.8.42 suffering from ulcerative gingivitis. As a routine he was tested for sickling and this was found to occur. A complete clinical and haematological investigation followed. It was impossible to obtain any history of previous illness from him as he spoke very little English. His sclerotics were slightly pigmented and his spleen was easily palpable one finger's breadth below the costal margin.

*Blood Picture*—R.B.C. 3 100 000 per c.mm. haemoglobin, 69 per cent. colour index, 1.1 W.B.C., 5,200 per c.mm. polymorphonuclears 40 per cent. lymphocytes 37 per cent. monocytes, 9 per cent eosinophils, 14 per cent.

*Sternal Bone Marrow*—Polymorphonuclears 40 per cent. lymphocytes, 4 per cent. monocytes 2 per cent. myeloblasts 0.6 per cent. eosinophils 5.4 per cent. neutrophil myelocytes 9.4 per cent. eosinophilic myelocytes, 2.2 per cent. neutrophil metamyelocytes 13.6 per cent. eosinophilic metamyelocytes, 2.6 per cent. plasma cells, 0.4 per cent. proerythroblasts, 0.6 per cent. normoblasts type A, 0.6 per cent. normoblasts type B 5.2 per cent. normoblasts type C 13.4 per cent. megakaryoblasts nil. Ratio of myeloid cells to nucleated red cells, 7.3. Bone marrow moderately hyperplastic and showed some upset of normoblastic hyperplasia indicated by the production of large early eosinophilic forms.

No malaria parasites were found in either peripheral blood or bone marrow. Marmite therapy was commenced on 12.9.42. By this time his dental condition was much improved.

On 9.10.42 blood examination showed R.B.C., 3,400,000 per c.mm. haemoglobin 72 per cent. colour index, 1.03 W.B.C., 5,000 polymorphonuclears, 34 per cent. lymphocytes, 30.5 per cent. monocytes, 7 per cent. eosinophils, 23.5 per cent.

His eosinophilia was explained by the demonstration of ankylostome ova in his stool. A pre-operative course of 3,000 mg. of ascorbic acid was given and splenectomy performed on 17.10.42.

The spleen was enlarged, dark purple in colour and firm. The surface was smooth and free from adhesions. The cut surface showed no infarcts. There was some thickening of the capsule and trabeculae and a moderate amount of fibrosis with small areas of congestion and haemorrhage scattered throughout the organ.

**Histological Features.**—Considerable fibrosis and siderosis with some hyaline of the fibrous tissue. Advanced aetrial changes of vessels. Reticular spaces for the most part empty. Malpighian bodies distorted, the capillaries dilated and the corpuscular material widely separated. Corpuscles surrounded by zones of haemorrhage. Splenic pulp compressed and packed with sickle cells.

There were no immediate post-operative complications. Successive blood counts after operation gave the following results—

21.10.42.—R.B.C. 4,100,000 per c.mm. haemoglobin, 90 per cent. colour index 1.1 W.B.C. 9,000 per c.mm. polymorphonuclears, 50 per cent. lymphocytes, 41 per cent. monocytes 10.5 per cent. eosinophils, 15 per cent. No malaria parasites found.

2.11.42.—R.B.C., 4,700,000 per c.mm. haemoglobin, 91 per cent. colour index 0.88 W.B.C., 8,800 per c.mm. polymorphonuclears, 43 per cent. lymphocytes, 27.5 per cent. monocytes, 11.5 per cent. eosinophils, 18 per cent. No malaria parasites seen.

19.11.42.—R.B.C., 4,800,000 per c.mm. haemoglobin, 92 per cent. colour index 0.8 W.B.C., 8,800 per c.mm. polymorphonuclears, 51.5 per cent. lymphocytes, 24 per cent. monocytes, 10 per cent. eosinophils, 18 per cent. No malaria parasites seen. Reticulocytes, 4.2 per cent.

He was discharged to his unit on 25.11.42.

He reported to the hospital for a "follow-up" blood count on 12.12.42. Although he appeared to be in good health his temperature was found to be 103 F and he was readmitted. A blood film showed malaria parasites including all the developmental forms of *P. falciparum*.

**Blood Count.**—R.B.C., 4,500,000 cells per c.mm. haemoglobin, 83 per cent. colour index, 1.0 W.B.C., 8,000 per c.mm. polymorphonuclears, 23.5 per cent. lymphocytes, 39.0 per cent. monocytes, 31.5 per cent. eosinophils, 6 per cent.

He was treated with intravenous quinine to which he responded rapidly his temperature being normal 2 hours later and remaining so until he was discharged from hospital 10 days later. Only one injection of quinine was given of grains x and this was followed by quinine grama x by mouth t.i.d.

He was admitted to hospital with a second attack of malaria on 9.3.43 when developmental forms of *P. falciparum* were once more found in great numbers. At this time his blood count was R.B.C., 4,200,000 per c.mm., Hb., 85 per cent., W.B.C., 8,000 per c.mm. polymorphonuclears, 60.5 per cent. lymphocytes, 26 per cent. monocytes, 8.5 per cent. eosinophils, 6 per cent. He again responded rapidly to quinine.

One month later he came into hospital with chickenpox when his blood count was R.B.C., 4,700,000 per c.mm. Hb. 92 per cent. W.B.C., 9,000 per c.mm. polymorphonuclears, 50 per cent. lymphocytes, 19.5 per cent. monocytes, 12.5 per cent. eosinophils, 12 per cent.

### Commentary

Despite his hookworm infestation this man's blood picture was not typical of an anaemia entirely due to hookworm, and it was concluded that sickle

of the erythrocytes was responsible for his condition. The response of his anaemia to splenectomy was rapid and sustained over a period of 8 months, during which time no haematurics were exhibited. No specific treatment for his hookworm infestation was undertaken.

Histologically, his spleen showed the characteristic stuffing of the splenic pulp with sickle cells and haemorrhages around the Malpighian bodies.

Although at the time I thought that this case was primarily an instance of sickle anaemia complicated by hookworm infestation, I am now of the opinion that this man's anaemia was of a heterogeneous variety viz a macrocytic hypochromic anaemia due to hookworm infestation and a dietary deficiency (dual or dimorphic anaemia TROWELL, 1943) with the addition of sickle anaemia. This conclusion is based mainly on the absence of a neutrophil leucocytosis and of conjunctival jaundice.

The main feature of his post-operative attacks of malaria was a fever accompanied by hardly any constitutional disturbances, in spite of blood films which showed a large number of malignant tertian malaria parasites, including developmental forms. His response to quinine was on each occasion rapid and dramatic. Since there is considerable evidence that quinine itself is a factor in the precipitation of blackwater fever in Europeans and Africans, it is of interest to note that despite the combination of malignant tertian malaria sickling of the red cells and the exhibition of quinine, he did not develop haemoglobinuria. His frequent post-operative attacks of malaria with an excessive number of malaria parasites in the peripheral blood illustrates the essential part played by the spleen in the suppression of malaria.

## GROUP II

### SICKLAEMIA IN ACUTE TOXIC CONDITIONS

#### Case 5

Pte. RICH, a native of the Cameroons, aged 18 years, was admitted to a casualty clearing station on 2.11.42, with delirium and photophobia. He was found to have head retraction and a positive Kernig. His conjunctivae were oedematous. Turbid C.S.F. was withdrawn and he was given sulphapyridine by mouth. A second lumbar puncture on 4.11.42 showed the fluid less turbid. On 6.11.42 he was transferred to hospital. By this time he had had 30 grammes of sulphapyridine. On admission he appeared very ill and emaciated. Temperature 97.6° F. conjunctivae oedematous and injected. Photophobia intense. Towards evening he became restless and noisy. Lumbar puncture C.S.F. turbid and red tinged with a blood stained clot. Cell count (approximate only due to clot) red cells, 2,850 per c.mm. leucocytes, 130 per c.mm. i.e. ratio of white cells increased. The red cells were sickle cells. Leucocytes were polymorphonuclears, 98 per cent., monocytes and lymphocytes, 2 per cent. culture sterile.

Blood Count.—R.B.C., 2,450,000 per c.mm. haemoglobin, 55 per cent. colour index, 1.1 W.B.C. 24,000 per c.mm. polymorphonuclears, 80.5 per cent. lymphocytes, 8.0 per cent. monocytes, 9.5 per cent. eosinophils, 1 per cent. myelocytes, 0.5 per cent. metamyelocytes, 0.5 per cent. Six nucleated red cells per one leucocyte. Majority of immature red cells were normoblasts type C with one or two normoblasts type B. Much anisocytosis. Sickling *in vitro* and *in vivo* demonstrated.

Sternal Bone Marrow.—Polymorphonuclears, 12.4 per cent. lymphocytes, 3.0 per



cent. monocytes, 1.4 per cent. eosinophils, 0.6 per cent. myeloblasts, 0.4 per cent. myelocytes, 5.6 per cent. metamyelocytes, 8.2 per cent. proerythroblasts, 5.4 per cent. normoblasts type A, 7.2 per cent. normoblasts type B, 20.6 per cent. normoblast type C, 32.2 per cent. megaloblasts, nil plasma cells, 2.2 per cent. megakaryocytes 0.4 per cent. Bone marrow showed intense normoblastic hyperplasia with normoblast of the large early eosinophilic type. Ratio of myeloid cells to nucleated red cells, 9:1.

*Lass den Bergh Reaction*—Weak direct and indirect reactions. Serum bilirubin 1.5 mg per cent.

His temperature which had risen soon after admission persisted at a level of  $102^{\circ}$  to  $103^{\circ}$  F. On 18.12.42 he became restless and irritable and semiconscious. He developed bilateral ophthalmoplegia and died the next day.

#### *Summary of Autopsy Report*

Body wasted. Conjunctivae jaundiced. Heart normal. Lungs congested. Thyroid suprarenals and pituitary normal. The spleen consisted of two dark fibrotic waxy masses, each about the size of a walnut: the two masses were connected by a desmoid of adhesions. Stomach and intestines normal. Liver slightly enlarged, pale on section and yellow stained. Kidneys small and congested. Brain convolutions flattened. Meninges congested. Two small patches of greenish exudate  $\frac{1}{2}$  inch in diameter on the right hemisphere near midline. Other small patches of exudate over the frontal lobes along both Sylvian fissures, over the optic thalami, and over the upper surface of the right cerebellum. Lateral ventricles slightly dilated. The cerebral cortex showed softening, especially of the right. A thrombus was present in the cavernous sinus on both sides. Smears of the exudate showed numerous polymorphonuclears, scanty lymphocytes and monocytes, and numerous Gram positive diplococci with the morphological characters of the pneumococcus.

#### *Summary of Histological Reports*

*Cerebrum* Capillaries engorged and plugged with agglutinated sickle cells. Perivascular necrosis associated with the thrombosed capillaries and some haemorrhage. Perivascular infiltration with inflammatory cells absent. In a few areas a fine monocyte infiltration. Sclerotic changes with hyalinization seen in walls of many vessels. Splenic capsule thickened and hyalinized. Splenic architecture lost and replaced by dense fibrous tissue. Malpighian bodies transformed into hyaline whorls of fibrous tissue containing a centrally placed vessel with narrow lumen and walls showing advanced sclerotic changes. Splenic pulp compressed and composed of a mass of reticulum packed with sickle cells, round cells and plasma cells. Giant cells containing pigment seen throughout the atrophic pulp.

#### *Commentary*

I believe this case to be an example of latent sickle cell anaemia precipitated into an active phase by the toxæmia resulting from pneumococcal meningitis. This led to focal necrosis and haemorrhage of the cerebrum and to a cavernous sinus thrombosis. Many lesions and disorders of the central nervous system have been attributed to sickle cell anaemia. These include thrombosis of the dural venous sinuses and pial veins, haemorrhagic infarction, cerebral necrosis, haemorrhage into the subarachnoid space, and hemiplegia and convulsions (WALKER and MURPHY 1941, BRIDGES 1939). These lesions are believed to result from the thrombotic tendency of sickle cells.

The small hard nodular shrunken dull grey spleen buried in a mass of fibrous adhesions is certainly a characteristic finding in a patient that has gone through several attacks of active sickle cell anaemia (DIOGA, 1935).

## Case 6

Spr AKANNI a Nigerian soldier aged 20 years, was admitted to hospital on 26 10 42. His only complaint was of fever of one day's duration. Temperature, 104.8° F. confused and irritable. Chest signs of bronchitis. Lumbar puncture opalescent blood tinged fluid not under pressure. 40 lymphocytes per c.mm. R.B.C., 2,200 per c.mm. chlorides 750 mg per cent. globulin, a faint trace. culture, sterile. Abdomen liver and spleen enlarged.

The patient was put on to sulphapyridine of which he was given a total of 22 grammes. The drug was discontinued on 31 10 42. By this time his condition had improved. His temperature was normal in the mornings but still showed a rise to 101° to 103° F at night. He became slightly jaundiced. For the next few days his general condition remained much the same apart from some deepening of his jaundice.

On 3 11 42 his evening temperature rose to 105.6° F and it was noticed that his abdomen was distended and that there was an appreciable enlargement of his liver and spleen.

On 5 11 42 his evening temperature was still up to 105.6° F. Very drowsy and confused, and complaining of thirst and headache. Liver and spleen still further enlarged.

On 5 11 42 he died.

*Investigations.*

*Urine*—28 10 42. Few leucocytes and scanty red cells. No ova, no casts. Culture sterile.

*Faeces*—Soft, dark, containing a few red cells and leucocytes. No amoebae or amoebic cysts. No pathogens isolated on aerobic culture.

*Blood*.—7 11 42. R.B.C. 3 200 000 per c.mm. haemoglobin 66 per cent. colour index, 1.0 W.B.C. 10 000 per c.mm. polymorphonuclears, 66 per cent. lymphocytes 16 per cent. monocytes, 14 per cent. eosinophils, 2.5 per cent. metamyelocytes, 1.5 per cent.

Classical sickling of over 70 per cent. of the erythrocytes.

No malarial parasites found in thick or thin smears.

*Summary of Autopsy Findings*

Body of wasted adult. Conjunctivae jaundiced. Cardio-vascular system, no abnormality. Hypostatic congestion of lungs. Liver enlarged to 3½ inches below costal margin, capsule under tension. On section, pale yellow-stained tissue showing early fatty degenerative changes. No evidence of amoebic hepatitis. Stomach and small intestines normal in appearance. Large intestine no congestion, ulceration or other abnormality found affecting the caecum, ascending colon, descending colon or rectum. The transverse colon in the region of the splenic flexure was firmly adherent to the spleen and a perforated cleanly punched-out ulcer approximately 1 inch in diameter was found. The edges of the ulcer presented no macroscopic sign of active inflammation and were not indurated. This ulcer communicated directly with the splenic pulp which was necrotic. No evidence was found of a leak into the peritoneal cavity. *Spleen*.—Enlarged five times and was of a peculiar spheroidal shape, firmly adherent by dense bands of fibrous tissue to the adjacent viscera and especially to the splenic flexure of the colon, and to the parietal peritoneum. Section showed that the ulcer communicated directly with the splenic pulp which was colliquative and necrotic, and of a foamy and poriferous appearance. Microscopic examination of the necrotic material revealed no amoebae. *Central Nervous System*.—Meninges congested. No exudate, cut section of brain showed focal capillary congestion.

*Summary of Histological Findings*

*Spleen*.—Intensely congested with multiple infarcts, spreading oedema, colliquative necrosis and gaseous emphysema. Arterioles showed advanced sclerotic changes. Reticulum increased and pulp packed with sickle cells and necrotic phagocytes. One large infarct communicating directly with lumen of colon. Muscular layers of gut gummed to splenic capsule by fibrous tissue. *Colon*.—Non-specific changes surrounding ulcer. No evidence of amoebiasis or tuberculosis.

*Commentary*

This man had a meningitis the toxæmia of which precipitated a sick cell crisis.

It is difficult to explain the mechanism that led to the formation of the intestinal ulcer and to its subsequent perforation into the spleen. Enlargement of the spleen may have occurred during a sickle cell crisis with infarction, the adjacent part of the gut then becoming adherent to the patch of perisplenic thrombosis due to impaction of agglutinated sickle cells in the capillaries of this region of the gut causing ulceration with subsequent perforation into the splenic infarct would then account for the necrosis of the spleen. The punched out character of the ulcer suggests a vascular cause, either thrombosis or spasm, leading to necrosis.

No amoebæ were found in the faeces or in the necrotic material of the spleen, and no evidence of amoebic ulceration of the gut was found at autopsy.

*Case 7*

Pts. S. B., a Gambian, was admitted to hospital on 8.4.42 with scabies and infected abrasions of both feet. A fortnight later he developed a pyomyositis in the muscles of his left axilla and his temperature rose to 101 F. Four weeks later he developed hæmoglobinuria which was preceded by a rigor and a sudden rise in temperature. Twenty-four hours later he became jaundiced and vomited "coffee ground" material. His spleen at this time was enlarged 1½ inches below the costal margin. The urine was a dark port wine colour with a pink foam, a heavy deposit of sediment and a cloud of albumen. Absorption bands of hæmoglobin were seen on spectroscopy and microscopic examination revealed a few red cells of sickle shape, a few casts and many leucocytes. No malarial parasites were found in the blood after repeated examinations. The blood serum showed a severe degree of hæmoglobinaemia. His blood picture on the day of onset of hæmoglobinuria was R.B.C., 1 160 000 per c.mm., hæmoglobin, 28 per cent., colour index, 1.1 W.B.C., 40 000 per c.mm., polymorphonuclears, 64.25 per cent., lymphocytes, 12.75 per cent., monocytes, 20.25 per cent., eosinophils, 0.5 per cent., metamyelocytes, 2.25 per cent. Some of the monocytes contained engulfed red cells. The red cells showed much anisocytosis and normoblasts, both intermediate and late forms were present in large numbers. Sickling of the red cells both *in vitro* and *in vivo* was demonstrated.

The patient was given alkali and intravenous glucose-saline and oxygen. A blood transfusion had to be abandoned after only 60 c.c. had been given as he developed a severe reaction.

Two days later his blood picture was R.B.C., 910 000 per c.mm. W.B.C., 90 000 per c.mm., polymorphonuclears, 65 per cent., lymphocytes, 17.6 per cent., monocytes, 12.0 per cent., eosinophils, 0.4 per cent., metamyelocytes, 2.8 per cent., myelocytes, 2.0 per cent., plasma cells, 0.2 per cent.

The sternal bone marrow showed an intense hyperplasia affecting both white and red cell series. No megaloblasts were present and no malarial parasites were seen.

At this time his spleen was no longer palpable, he became unconscious and developed Cheyne-Stokes respirations. A hæmorrhage was seen in the right fundus, and his feet and ankles, the dorsum of his hands and penis became oedematous. Death occurred 5 days after the onset of hæmoglobinuria. During these 5 days his urine showed a progressive decrease in hæmoglobin content and an increase in the number of red cells and leucocytes. The serum was examined by Lt.-Col. B. G. MARSHALL and Brig. G. M. FINDLAY who demonstrated no reduction in the *in vitro* inhibitory hæmolytic factor.

*Summary of Autopsy Findings*

Body of wasted young adult. Conjunctivæ and subcutaneous tissues jaundiced. A firmly walled abscess in the muscle of the left axilla (pyomyositis). Lungs oedematous and water-logged. Stomach congested, no petechial hæmorrhages. Intestinal mucosa

congested. Spleen enlarged to twice normal size, congested and showing two wedge-shaped haemorrhagic infarcts. Liver congested and yellow stained with patchy fatty degeneration and necrosis. Kidneys congested and dark in colour surface smooth and capsule stripped easily. Brain pale and oedematous. Cut surface showed advanced softening of both cerebral hemispheres.

#### *Summary of Histological Findings*

**Liver**—Degenerative changes with necrosis of the peripheral and central type accompanied by marked congestion and much separation of the liver cell columns. Blood in the distended vessels and sinusoids mostly laked but a few sickle cells could be made out. Torres inclusion bodies and Councilman lesions not demonstrated. **Spleen**—Destruction of the parenchyma and stuffing with sickle cells of the infarcted areas. In other parts the pericorpuscular zones of the Malpighian bodies surrounded by haemorrhage. Much separation of the cells of the Malpighian corpuscles with dilatation of the capillaries and haemorrhage into the surrounding tissue. An early fibroblastic response with a diffuse round cell infiltration possibly a result of the damage by haemorrhage to the Malpighian bodies. Sinusoids mostly empty and the pulp stuffed with sickle cells and immature red cells. **Kidney**—Congestion of the glomerular capillaries. Degenerative changes affecting the tubules with desquamation of the renal epithelium. The second convoluted tubules appeared collapsed and separated by a fine oedematous interstitial tissue. Blood vessels congested and packed with sickle cells. **Cerebrum**—Majority of the capillaries blocked with autolytic thrombi formed of fused sickle cells, and surrounded by a fine monocytic infiltration. In some vessels an endothelial proliferation with hyalinization was demonstrated. Nerve cells showed a varying degree of degeneration associated with the blocked vessels and shading down to complete necrosis in one large area.

#### *Commentary*

The differential diagnosis lay at first between yellow fever blackwater fever and sickle cell anaemia in an intensely acute haemolytic crisis. Yellow fever was ruled out as it does not show haemoglobinuria and was furthermore excluded at autopsy by the histological appearances of the liver. Although haemolytic crises occur in sickle cell anaemia, they are not usually sufficiently severe to produce haemoglobinuria. The sudden onset of haemoglobinuria with rigors, fever and an enlarged spleen, followed by jaundice are typical of blackwater fever but the presence of many sickle-shaped erythrocytes and leucocytes in the urine were the unusual feature. Serial examinations of the urine showed a progressive diminution in the amount of haemoglobin and an increase in the number of red cells and leucocytes. The titre of the tissue inhibitory haemolytic factor in the serum was not reduced, as it is in blackwater fever (MAEGRAITH FINDLAY and MARTIN 1943) and sections of the spleen showed the histological features characteristic of the sickle cell anomaly (RICH 1928). The changes found in the organs were those due to thrombosis haemorrhage and consequent necrosis, features associated with sickle cell anaemia. There was an intense marrow hyperplasia reflected in the peripheral blood by the appearance of immature red cells in moderate numbers at an early stage of the haemoglobinuria—a finding more characteristic of sickle cell anaemia in an acute haemolytic phase than of blackwater fever. It is however difficult to decide whether the toxæmia due to the pyomyositis precipitated an attack of acute sickle cell anaemia followed by intense haemolysis or that as a result of an attack of blackwater fever the characteristic features of sickle cell anaemia were superimposed.

## Case 8.

Pte DANFA, a Gambian, aged 27 years, was admitted to hospital on 8.12.42 with diarrhoea and vomiting. He gave a history of cough with sputum for 2 months, and of pain in the limbs of 3 days duration. His temperature was 99.4 F., and he had ulcerative gingivitis. He was extremely emaciated. His chest was clear on clinical and X-ray examination. He was tender in the left hypochondrium but no enlargement of his spleen could be felt. *Sputum*.—Muco-purulent, no tubercle bacilli found. *Faeces*.—Soft and no blood or mucus. *B. coli* only isolated. *Blood Count*.—R.B.C., 2,410,000 per c.mm. haemoglobin 50 per cent. colour index, 1.0 W.B.C. 11,000 per c.mm. polymorphonuclears, 83 per cent. lymphocytes, 14 per cent. monocytes, 22.5 per cent. eosinophils, 0.5 per cent. Red cells were shown to sickle in *in vitro* and *in vivo*. Further blood investigations carried out while in hospital were as follows:—

18.12.42.—R.B.C., 2,000,000 per c.mm. haemoglobin, 43 per cent. colour index, 1.07 W.B.C., 12,600 per c.mm. polymorphonuclears, 55.3 per cent.; lymphocytes, 20 per cent. monocytes, 24.5 per cent.

30.12.42.—R.B.C., 900,000 per c.mm. haemoglobin, 24 per cent.; colour index, 1.2 W.B.C., 23,000 per c.mm. polymorphonuclears, 74 per cent., lymphocytes, 9.5 per cent. monocytes, 16.5 per cent. eosinophils, 0 per cent. Normoblasts type B and C present in moderate numbers. Many monocytes contained ingested red cells.

*Sternal Bone Marrow*.—Polymorphonuclears, 25.6 per cent. lymphocytes, 0.3 per cent. monocytes, 6.8 per cent. eosinophils, 0.4 per cent. myeloblasts, 0.4 per cent. neutrophil myelocytes, 4.4 per cent. neutrophil metamyelocytes, 6.0 per cent. eosinophil metamyelocytes, 0.4 per cent. plasma cells, 2.8 per cent. proerythroblasts, 3.2 per cent. normoblasts type A, 9.2 per cent. normoblasts type B, 22.5 per cent. normoblast type C, 16 per cent. megakaryocytes, 1.3 per cent. megakaryoblasts, nil. An extremely hyperplastic marrow.

In spite of dental treatment for his gingivitis, large doses of iron and restimulants and blood transfusion, his general condition deteriorated rapidly and he contracted Flexner dysentery and died on 9.1.43.

*Summary of Autopsy Findings*

An extremely emaciated body. Conjunctivae jaundiced. Extensive pyorrhoes and ulcerative gingivitis. Heart small and flabby. Stomach and intestines normal. Liver normal in size, cut surface firm and blurred. Spleen small in size, weighing only 79 grammes, wrinkled and lobulated by scar tissue and bound to surrounding structures by dense adhesions, cut surface entirely fibrosed and dry. Suprarenal small weighing only 3 grammes and only 2.5 mm. in thickness, cut section showed cortical zones diminished in size. Kidneys normal size, cut surface pale with glomerular congestion. Mesenteric pale. Brain oedematous and section showed early softening and degeneration of its anaemic type.

*Summary of Histological Findings*

*Spleen*.—Capsule thickened and irregular in outline. Much fibrosis with loss of splenic architecture. Pulp reduced to a mass of reticulum packed with sickle cells. Malpighian bodies ill defined and represented by a central arteriole and concentric laminated degenerate whorls of sidero-fibrotic tissue. Vessels showed marked sclerotic changes. Sinusoids compressed and empty. *Lung*.—Chronic emphysema. *Cerebrum*.—Vessels congested and packed with sickle cells, many containing masses of agglutinated sickle cells. *Suprarenal*.—Vascular system congested and packed with sickle cells. Length of cell columns reduced, zona glomerulosa almost completely absent, and zona fasciculata and reticularis much diminished in size from necrosis of cells. Cortex in one pole of the gland completely destroyed by haemorrhage.

*Commentary*

This is believed to be a case of Addison's disease associated with sickle-cell anaemia and complicated by Flexner dysentery.

The main features of the autopsy were the fibrosed and shrunken spleen,

the capillary thrombosis and softening of the cerebrum, and the degeneration and necrosis of the adrenal glands with loss of cortex.

The diagnosis of Addison's disease was not made during life and unfortunately there is no record of the blood pressure in this case. It was thought that an increasing haemolytic anaemia with leucocytosis and a hyperplastic bone marrow containing no abnormal cells occurring in a patient whose red cells had been shown to sickle *in vitro* and *in vivo* was sufficient evidence to warrant a diagnosis of sickle cell anaemia. Low blood pressure has been noted in sickle cell anaemia, but the findings in this case suggest that a concomitant affection of the adrenal glands may well be an occasional explanation of this sign. It may be suggested that the gland in this instance was destroyed by thrombi formed from agglutinated sickle cells and haemorrhage during recurrent crises of sickle anaemia, but I consider it more reasonable to assume that the adrenal atrophy was caused by extra vascular factors e.g. a toxin which in turn precipitated an acute sickle cell crisis.

#### DISCUSSION

The demonstration of sickle cells either in fresh blood preparations or in formal fixed tissues is pathognomonic only of sicklaemia. A diagnosis of sickle cell anaemia based on the association of anaemia with sicklaemia is unjustified as factors other than sickling of the red cells may be responsible for the anaemia. As in Cases 1, 2 and 3 a sickle cell haemolytic crisis may well be initiated by an anaemia of different aetiology. When this occurs the signs and symptoms of sickle-cell disease are superimposed and the nature of the underlying anaemia may be masked. The type of anaemia must be fully investigated before it can be attributed to sickling alone and the value of bone marrow examinations obtained by sternal puncture has been repeatedly shown during the course of this paper. Cases included in Group I illustrate the importance of this examination revealing an anaemia due primarily to other causes.

The bone marrow during a sickle cell crisis shows an intense erythroblastic and leucoblastic hyperplasia with considerable increase in the number of monocytes many of which may contain engulfed red cells. The normoblasts tend to be large, early eosinophilic forms with a well patterned nucleus such as are found in haemolytic anaemias and in conditions of prolonged loss of peripheral blood. The diagnosis of a sickle cell haemolytic crisis will therefore be suggested by an anaemia with leucocytosis jaundice and a hyperplastic bone marrow which shows no abnormal cells, occurring in a patient whose red cells have been shown to exhibit sickling *in vitro*. It is then confirmed by the demonstration of sickle cells *in vivo*.

Cases in Group II are examples of sickle cell crises precipitated by other more urgent diseases. The conditions responsible in this series appear to be tropical myositis and blackwater fever meningitis and Addison's suprarenal disease.

In two of these cases (Cases 5 and 8) the spleen at autopsy presented as a small, hard, nodular dull grey organ. Cut sections showed thickened trabeculae and yellowish brown nodules scattered throughout the pulp—the result of organization of haemorrhagic areas (DIGGS 1935). The pulp itself had been transformed into a reticular mass packed with sickle cells. These extensive, degenerative changes may be taken as evidence of frequent sickle cell crises that had occurred in the past.

Lesions found in other organs are fundamentally similar to those that have been described in the spleen, namely tissue destruction by haemorrhage and thrombosis followed by necrosis. The thrombi are formed from agglutinated sickle cells which are impacted in the smaller vessels. Any organ may be involved and the large number of symptoms that have been associated with sickle cell anaemia is therefore not a matter for surprise. These observations suggest that the chief feature of the condition is not the anaemia but the liability to thrombosis that occurs during a sickle cell crisis. The term "sickle cell disease" would therefore appear to be preferable to "sickle cell anaemia".

As a sicklaemic subject is always apt to develop an acute haemolytic crisis, I would emphasize the necessity for all coloured patients admitted to hospital to be tested for sickling. From my observations this would appear to be of special importance in acute toxic conditions.

### SUMMARY

Eight cases of anaemia associated with sicklaemia occurring in West African natives are recorded. The difficulties in diagnosis are discussed and the need for a complete haematological investigation is stressed. Toxic conditions as precipitating factors in the production of sickle cell haemolytic crisis are listed and the importance of "testing for sickling" in acute diseases is emphasized. It is suggested that liability to thrombosis from impaction of agglutinated sickle cells is of greater importance than the presence or absence of an anaemia and that the term sickle cell disease would be therefore preferable to that of sickle cell anaemia.

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## STUDIES ON THE WELTMANN REACTION IN MALARIA CASES

BY

J. KLEEBERG M.D. (BONN)

*Head of the Medical Department A Hadassah-Rothschild University Hospital,  
Jerusalem Palestine*

The literature of tropical medicine cites a number of blood reactions mostly globulin reactions which indicate certain groups of diseases rather than being pathognomonic for one alone (BRAHMACHARI 1917 CHORINE 1938 Formol-gel reaction HENRY RAY 1931 SIA 1921 and 1924 WOLFF 1939). The Weltmann test is also a globulin reaction but it is not at all specific for malaria or any other disease. However I shall attempt to point out its advantages.

In 1930 WELTMANN described a simple coagulation reaction (W.C.R.) of blood proteins by calcium chloride. This unspecific test makes it possible according to its discoverer to distinguish between acute inflammatory and chronic proliferative processes.

Only a few articles followed in the German literature concerning the general clinical significance of this coagulation test. In America since 1938-1939 LEVINSON has made extensive studies on the subject. In the American literature papers appeared on the W.C.R. in the fields of pediatrics, neurology and tuberculosis. Together with Dr. UHNA and Dr. ENNISON we have used the W.C.R. during 3 years on about 1 000 patients. We can confirm the results outlined by WELTMANN himself, LEVINSON and others, concerning its general clinical importance.

In England the reaction is hardly used and in tropical medicine the standard books of CRAIG and FAUST, MANSON BARR, ROGERS, MEGAW and STRONG-STITT do not mention the Weltmann reaction. I have so far been able to find only three articles from 1930 until now dealing with W.C.R. and malaria. There is LANDEIRO (1935) whose paper



is available in Portuguese only. More recently LEVINSON and McFATE (1943), in their *Clinical Laboratory Diagnosis* mention malaria (among the detailed schemes for other diseases). A Bulgarian author TSCHILOW (1931) examined forty cases of malaria in 1931. His results are the same as mine.

However I thought it worth while 13 years after this apparently forgotten paper to report my studies in twenty five cases of malaria because they confirm the results of TSCHILOW and of LEVINSON because the reaction seems to me of diagnostic value, as indicating the possibility of a haemolytic process and because I believe this simple reaction might become an interesting test in the field of tropical medicine in general.

The technique as published by LEVINSON and McFATE (1943)\* is as follows

1. Stock calcium chloride solution. Dissolve in water 50 grammes of dry crystals of reagent  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  and dilute to 500 c.c. in a volumetric flask.

2. Dilute calcium chloride solutions. Transfer 5 c.c. of stock calcium chloride solution to a 500 c.c. volumetric flask and dilute to 500 c.c. with water. Mix and transfer to a bottle numbered "1". In turn transfer 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0 and 0.5 c.c. of stock solution to the volumetric flask and dilute each to 500 c.c. Mix and transfer to bottles numbered 2 to 10 respectively.

#### Apparatus.

1. Metal test-tube racks, to hold 13 x 100 mm. test-tubes.
2. Boiling water bath.

#### Procedure

Place ten test tubes, 13 x 100 mm. in a metal test tube rack and number consecutively from 1 to 10. Into each tube pipette 5 c.c. of the similarly numbered solution of calcium chloride. Then add 0.1 c.c. of the haemoglobin free serum. Shake the mix so the contents will be mixed and place in a boiling water bath for 15 minutes. Remove from the bath and read. The contents of the tubes may be clear faintly opalescent, turbid or there may be flocculation. There is usually a sharp and easily noted difference between flocculation and turbidity.

The number of the tubes in which flocculation occurs was designated by WELTMANN as the coagulation band (C.B.). If there is very slight or doubtful flocculation in one tube a rare occurrence, the reaction is interpreted as being intermediate between that tube and one before it and is designated by the figure  $\frac{1}{2}$ .

The blood should be taken in the morning, before breakfast, though a cup of coffee or tea or even a rush does not alter the result. Serum after a heavy meal cannot be used, nor can haemolytic serum be used.

SCHWEINBURG and EVANS (1941) emphasize that there should always be one normal serum to test as a control. The bottles must be protected from the  $\text{CO}_2$  of the air by keeping them thoroughly closed. The stock solution can be kept for a very long time the dilutions should be discarded after 1 month. The reading of the coagulation tube must be made immediately after removal from the water bath.

\* I thought it useful to give details of the procedure, because at the present time it may be difficult to get the original literature.

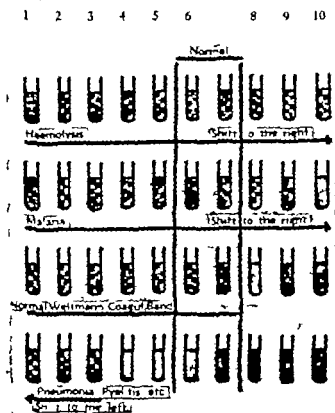
### Interpretations

In normal serum, the first six tubes usually show flocculation. Sometimes there is slight or doubtful flocculation in the seventh tube. The normal coagulation band is therefore 6 to 6<sup>+</sup> and remains remarkably constant in a serum of a healthy person.

The illustration shows the technical arrangement. Coagulation values of 6 or 7 are "normal," and higher numbers (8, 9, 10) means a "shift to the right" or a prolonged coagulation band (C.B.).

Vice-versa, values 5-1 indicate a "shift to the left," or a shortened coagulation band.

WELTMANN and his co-workers drew two conclusions: the WELTMANN Coagulation Reaction (W.C.R.) has a general and a specific significance. "Shift



to the left or shortened C.B. indicates an acute inflammatory exudative process. Shift to the right or lengthened C.B. is found in chronic proliferative process.

Concerning the *specific* importance, WELTMANN has described in several papers how all patients with liver-cirrhosis showed a prolonged C.B., indicating a toxic damage of the liver-cells as is usually the case in hepatic cirrhosis.

### GENERAL APPLICATION OF W.C.R.

This simple method was employed by us in twenty five cases of malaria. We can distinguish three types of reactions: thirteen cases showed a definite

shift to the right, figures like 8 or 9 (prolonged C.B.) ten cases gave abnormal C.B. (figures like 6 or 7) only two cases showed a shift to the left (4 or 5).

The W.C.R. in cases of acute infectious diseases is nearly always more or less shifted to the left. This is the experience of many investigators (WELTMANN, LEVINSKY) and our own results in about 1000 cases are similar (KLEIN, EINHORN and UNNA). We should have expected, especially when patients had high fever W.C.R. with figures like 4 or 5 if not even less (2 or 3) from this point of view the group with numbers such as 6 or 7 is surprising. We may say that twenty three out of twenty five acute malaria cases give an unexpected result with W.C.R., a so-called "normal range," or even a definite shift to the right (ten cases). The real prolongation of the coagulation band and the "masked" one (the group with figures like 6 or 7) did not depend either on the type of the malarial parasite or on the level of the temperature. The results were the same during an attack with chill and fever and on days between the attacks (*i.e.*, tertian malaria). No relation has been established with the accompanying anaemia.

The chemical composition of the blood proteins of a healthy person under normal conditions is very constant (BEST and TAYLOR, KECWICK and MCFARLANE). On the other hand diseases, especially those with high fever rapidly change the ratio albumin globulin (KECWICK and MCFARLANE) as the total protein or the globulin fraction alone. (SNAPPER, 1943)

Nearly all of the previously mentioned tests are based upon hyperglobinaemia. Thus the Henry test was simplified by CHORINE by using distilled water the original Henry technique of adding melanin from the eye of an eel merely makes the flocculation more easily visible, but it is not essential. (STRONG-STITT 1942.) According to MEGAW both Henry's and Chorine's tests are globulin reactions and not specific for malaria. Likewise the modification by WOLFF (1939) with buffers, is unspecific. (STRONG-STITT) We know that the formol-gel test, discovered by GATÉ and PAPACOSTAS (1920) is positive in all cases of hyperglobinaemia. Therefore positive flocculation may be obtained in cases of malaria, kala-azar subacute bacterial endocarditis, trypanosomiasis, schistosomiasis, even in cases of septicaemia. (KYLIN 1938 LEVINSKY and MCFARLANE, 1943 STRONG STEIN WORTHEIMER). ROGERS (1942) cites LEVY who says that all formalin tests depend on an excess of altered euglobulin and a decrease of serum albumin specially in the blood in kala-azar. The alkylol test, modified by NAPIER, has its value for chronic cases in kala-azar in certain places, where some of the above mentioned diseases do not enter into consideration in differential diagnosis, or at least not within the first months of the disease.

The W.C.R. belongs to this unspecific group of globulin-flocculation, most probably the increase of globulin being the main cause. The main production place of globulin formation and transformation is the liver (WHIPPLE).

WELTMANN always held to his opinion that, in all toxic liver cell damage, the C.B. is prolonged and shifts to the right as in certain cases of jaundice and always in cirrhosis. There is liver damage in malaria infection by the invading parasites which is either anatomical (BOYD FAIRLEY 1937 SMITH and GAULT STRONG-STITT, 1942) or functional (OU 1941 ORLINA 1941 MANSON BAHR, 1940 KOPP and SOLOMON 1943)

We had no cases of pregnancy in malaria where according to WICK-RAMASURIYA (1937) the cloudy swelling and the fatty degeneration is very pronounced. I suppose the W.C.R. in such cases might give quite interesting results. However if we do not go into details on liver damage it is because I do not think that liver changes are the decisive factor for our unexpected results in the W.C.R. in malaria. It is difficult to explain such a coagulation result due to a parasitic liver invasion, compared with the opposite reactions of the C.B. in nearly all the other infectious diseases with high fever. There too the liver is highly involved (e.g. pneumonia) and the W.C.R. goes to the left. Then the rapidity with which the W.C.R. swings to the right even on the first day of chill and fever we found in several cases already results of 8 or 9. In 1931 WELTMANN already explained the results of TSCHILOW (1931) as a possible haemolytic reaction. He came to this conclusion as a result of his observation on cases of pernicious anaemia. I also believe that haemolysis is the most probable factor producing this peculiar coagulation reaction.

One can easily produce this phenomenon with every normal blood serum which gives a normal range of W.C.R. If one makes by any procedure such a serum haemolytic, the new coagulation test shows one or two tubes more to the right therefore clear unhaemolysed sera only are to be carefully examined. We have examined two cases with acute haemolytic anaemia due to sulphanilamide and dapsone. Both cases whose C.B. had been shifted to the left (surgical cases) swung during the haemolytic crises to 9. When the recovered patients left the hospital, they had W.C.R. 6. We have proved that the sulphanilamide group alone has no effect on the W.C.R.

We therefore think that it is the haemolytic effect of the malaria infection which causes the prolongation of the C.B. I should like to suggest that perhaps the number of the coagulation tubes, 8 or 9 or 10 or only 6 or 7 may be a quantitative indication of the degree of the haemolysis. If this holds good it may be a very helpful method in addition to or instead of reticulocyte counting (FAIRLEY) and especially in watching patients with threatened blackwater fever.

No satisfactory explanation can as yet be offered as to why two patients with malaria had a C.B. of 4 or 5. They had no other complications which could have changed the C.B. Other investigators and we, too have observed that patients, suffering from typhoid, for instance, had a C.B. of 5. As soon as they got any complications (broncho-pneumonia, thrombophlebitis, otitis cystica) the W.C.R. changed and swung to the left,

2 or 3 returning to its former level when the complication had passed away. As has been said, no such complications occurred in these two patients with malaria. We are commencing investigations on a larger scale and hope to discover the reasons for the exceptional results.

One case is worth reporting in detail. The patient, S., with tertian malaria, presented a coagulation band of 9. He received quinine arsen treatment and left the hospital after 8 days with no fever, no plasmodia, and in a satisfactory condition. After another 7 days he had to be re-admitted on account of high fever, headache, a sensation of burning in the eyes. A relapse was suspected but no plasmodia could be detected although the blood was thoroughly searched during 2 days. The coagulation band was 7 instead of 9 as it had previously been. The temperature chart and the positive Weil-Felix reaction after 10 days indicated that the patient had in the meantime contracted typhus of the mild murine type prevailing in this country. Thus the change in the W.C.R. had given a hint that the diagnosis should be revised.

All these facts show that in a given case of high acute fever the W.C.R. has a certain diagnostic value, especially as the result is obtainable on the very first day of the fever. Influenza, tonsillitis, pneumonia would give a marked "shift to the left" and even typhoid (not easily identified in the first few days) would not show a shift to the right. Typhoid mostly gives the figures "5" maximum "6". Only infectious jaundice might come in for special consideration as it has a W.C. + Reaction though probably for other reasons (liver damage).

As far as I could find in the available literature, the W.C.R. has not been used in the field of tropical medicine. Theoretically judging from what is known of the W.C.R. in other infectious diseases, one might expect in patients with schistosomiasis or kala-azar at the time of high fever a "shift to the left." Only when liver-cirrhosis has definitely set in a swing to the right should be obtained. But to examine the two mentioned, and other tropical diseases, by the Weltmann method may lead to another advantage. WELTMANN (1931) has pointed out the value of repeated W.C.R. in the course of a given disease in obtaining a prognostic view. Since the W.C.R. is unaffected by the height of fever or by any accompanying anaemia, the W.C.R. may sometimes be more helpful in prognosis than the sedimentation time. (WELTMANN LEVINSON KLEESBERG). Taking into consideration the very simple technique, may I again be allowed to urge that this Weltmann Reaction be tried in the field of tropical medicine.

#### SUMMARY

1. The technically simple procedure of the "Weltmann Coagulation Reaction" (W.C.R.) was employed by us in twenty-five cases of malaria.

2. Nearly always in acute infections with high fever the Coagulation Band (C.B.) is shortened and the W.C.R. is shifted to the left. But although

malaria attack clinically produces all the features of an acute inflammatory process, the W C R. is shifted to the right (thirteen cases) or stays normal (ten cases) Very similar results were obtained by Tschilow (1931)

3 The W C R. is an entirely unspecific protein globulin reaction, but considering all above mentioned facts it gives in tropical countries a certain hint in the diagnosis of acute fevers

4 It is suggested that haemolysis may be responsible for the occurrence of the lengthened C B in malaria.

5 The Weltmann test is particularly valuable in all haemolytic processes It might prove to be of special help in blackwater fever

6 A repeated W C R. in the course of a disease can, like the sedimentation time, be of help in prognosis Therefore interesting results might be obtained in other tropical diseases besides malaria.

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## A CONTRIBUTION TO THE STUDY OF MALNUTRITION IN CENTRAL AFRICA

### A SYNDROME OF MALIGNANT MALNUTRITION

BY

H. C. TROWELL, M.D., F.R.C.P.,\*

*Lecturer in Medicine Uganda Medical School Kampala Uganda,*

AND

E. M. K. MUWAZI

*Assistant Medical Officer Uganda Medical School*

The study of malnutrition in tropical Africa is at an elementary stage. At the present time it is passing through a phase of peculiar difficulty for on the one hand the classical diseases of malnutrition, such as pellagra beriberi scurvy and rickets have been but seldom reported whereas on the other hand surveys of African diets reveal that they are often deficient in many respects, notably in total calories protein vitamins of the A B and C groups depending largely on what are the basic articles of diet. There is thus a

\* Thanks are due to Dr D HARVEY for the estimations of the plasma proteins, and to Dr J SCOTT BROWN for the radiological examinations in the interpretation of both of these investigations we have offered our own personal opinion. Dr H. G. WILTSCHKO and Dr R. S. F. HENNINGSEN gave assistance in the pathological investigations and Mr HYON performed all the blood counts. Colonel D BELL and Lieut. Colonel A. KICKWICK allowed me to see their own nutritional survey of African soldiers. The Armour Laboratories and the Lederle Laboratories sent liver extracts for trial, so did the British Drug Houses, together with certain of the more obscure fractions of the vitamin B complex.

Thanks are due to the DIRECTOR OF MEDICAL SERVICES, UGANDA, for permission to publish this article.



serious discrepancy between those who are engaged in clinical work and who seldom diagnose malnutritional disease and those engaged in preventive medicine who state that the diets are gravely deficient in many essential respects.

At the same time an increasing number of papers have emerged which attempt to describe a new aspect of malnutrition. Though there is a striking agreement about the clinical picture yet the underlying pathological and biochemical changes have been but little investigated. The aetiology and the treatment are therefore uncertain.

### THE LITERATURE

The condition was first adequately described by CICELY WILLIAMS (1933, 1935, 1940) she called the condition "kwashiorkor" and considered that it was a new clinical entity. TROWELL (1937, 1940, 1941), influenced to a certain extent by the views of STANLEY (1934, 1936, 1944), considered that the disease was "infantile pellagra." SUZMAN (1942) and KATZ (1943) described the condition in the natives of South Africa. SCOTT BROWN and TROWELL (1944) described the radiological changes in the gastro-intestinal tract, and in this communication TROWELL stated that he could no longer consider the condition to be that of pellagra. GILLMAN and GILLMAN (1944), in a preliminary report of their liver biopsy results, to be reported shortly in other communications, demonstrated that the essential and earliest manifestation in the liver was that of fatty degeneration, from which recovery is extremely slow. In spite of a good diet, supplemented by large amounts of ascorbic acid, and thiamin. In their opinion certain of these cases proceeded to pigment cirrhosis. Their work was done in Johannesburg, and they were able to exclude the presence of tropical parasites, a point which has always troubled workers in the warmer parts of the world. In their opinion, demonstrated by serial liver biopsy, the liver responded quickly to the administration of desiccated hog's stomach.

### PERSONAL OBSERVATIONS (1943) 200 CASES.

Adults 144 fatal 25 improved 119

Children 56 fatal 10 improved 46

*Incidence*—This disease accounted for about 10 per cent. of the medical adult admissions and about 50 per cent. of the children.

*Age*—Cases were divided into two groups. Children (under 15 years) largely Ganda and Adults (over 15 years), largely immigrant labourers from Ruanda-Urundi. All cases were African natives.

#### Children.

7 months to 1 year	10 cases (none below 7 months)
1 to 2 years,	22 cases.
2 to 3 years,	16 cases.
3 to 5 years,	3 cases.
5 to 10 years,	2 cases.
10 to 15 years,	3 cases.

#### Adults

15 to 20 years	40 cases.
20 to 30 years,	52 cases.
30 to 40 years,	35 cases.
40 to 50 years	15 cases.
Over 50 years,	2 cases.

*Place, Climate and Endemic Diseases*—Observations were confined to Kampala, the capital of Uganda situated at a height of 3,800 feet. The cases

encountered among children came largely from the local Ganda tribes, to a less extent from the immigrant Ruanda Urundi tribes. Kampala is a hyper-endemic area of sub-tertian malaria, and a fair amount of quartan malaria in childhood. Almost every African baby contracts malaria in the first few months of life and by the 3rd month infection must be regarded to be almost universal in African children. Hookworm disease is very common in the humid climate but is seldom contracted before walking is begun in the 2nd year after which it increases in frequency until in adult life most Africans are infected but the infection to judge by postmortems is usually light being under fifty worms. Ascariasis and taeniasis are uncommon in Kampala bilharziasis is almost unknown trypanosomiasis is seldom seen filariasis (apart from onchocerciasis and the *perstans* varieties) is not seen. Kala azar does not occur. The blood Kahn is positive in approximately 50 per cent. of Ganda adults due presumably to syphilis for yaws is hardly ever seen. Babies are usually not weaned until well into the 2nd year and often the child is then restricted to the usual adult diet of cooked plantains, sweet potatoes and tea for meat, milk, green vegetables, beans, nuts, eggs or fish are seldom given to the children. Neither adults nor children take any cereal.

Cases in adult life are seen most commonly among the immigrant Ruanda Urundi tribes. These walk some 500 to 800 miles from the Belgian mandated territory of Ruanda Urundi to work on the European Indian and African estates and the peasant cotton plantations.

### NATIVE PEASANTS DIET

It is impossible to give any exact estimate of the content of the poor peasant's diet, but the following is a rough estimate of the daily intake and of its approximate composition.

1 *Very poor labourer's diet* 4 lb. of undried cassava daily (3 lb. when prepared for cooking) = 1750 calories, 10 grammes protein, fat nil, calcium 0.5 gramme, iron 4 mg., vitamin A nil, B<sub>1</sub> nil, riboflavin nil, nicotinic acid nil, vitamin C 450 mg. (reduced by cooking 1 to 2 hours)

2 *Immigrant labourer's diet* 5 lb. of sweet potatoes (3½ lb. after preparation) ½ oz. of meat, ½ oz. of green vegetables = 2,000 calories, 25 grammes protein, 1 gramme fat, 0.25 gramme calcium, 8 mg. iron, vitamin A 600 units, B<sub>1</sub> 1.0 mg., riboflavin 2.4 mg., nicotinic acid 1.0 mg., vitamin C 420 mg. (reduced by cooking 1 to 2 hours)

3 *Ganda peasant's diet* 8 lb. of cooked plantains (4 lb. after preparation) 2 lb. of potatoes, ½ oz. meat, 1 oz. green vegetables 1 oz. sugar (with tea) 1½ oz. groundnuts = calories 2,950 protein 47.0 grammes, fat 22.0 grammes, calcium 0.3 gramme, iron 14.2 mg., vitamin A 3,800 units, B<sub>1</sub> 1.1 mg., riboflavin 1.85 mg., nicotinic acid 7 mg., vitamin C 450 mg. (before cooking 1 to 2 hours)

4 *Children of the local tribes* are largely weaned on to diet 3 they have three meals a day.

It is considered that an adult African of 55 kg. engaged in moderately active work should receive calories 2,600 to 3,000 protein 60 to 70 grammes, calcium 0.8 gramme, iron 11 mg., vitamin A 5,000 units, vitamin B<sub>1</sub> 1.8 mg., riboflavin 2.2 mg., nicotinic acid 15 mg., vitamin C 75 mg. Certain of these amounts should be increased if long marches are undertaken by immigrant labourers.

It is therefore considered that —

1 Very poor labourer's diet is seriously deficient in everything except vitamin C

2 Immigrant labourer's diet is seriously deficient in calories, protein, calcium, vitamin A and nicotinic acid, and moderately deficient in iron and B<sub>1</sub>

3 Ganda peasant's diet is moderately deficient in protein, calcium and nicotinic acid, and slightly deficient in vitamin A, riboflavin and B<sub>1</sub>

### THE CLINICAL PICTURE.

The clinical picture has previously been described by CICELY WILLIAMS (1933) TROWELL (1937), SCOTT BROWN and TROWELL (1944) it consists of a gross loss of bodyweight in adults or a failure of growth in children, oedema, pallor of the skin and hair (Fig. 1), crazy pavement dermatosis (Fig. 2), loose stools steatorrhoea in childhood, macrocytic anaemia, slight mental and neurological changes and deficiency bowel pattern. It is not proposed to mention these points again in any detail, but to stress one or two aspects previously inadequately described.

Once the gross case of this syndrome is recognized, the question emerges what are the earliest signs of the syndrome? In childhood these are a failure to gain weight and some softness and brownness of the African hair and pallor of the facial skin. The hair changes are only visible if the head is washed so that in many African tribes this change would not be noticed. If this point is accepted then the prevalence of this form of malnutrition in African children of Uganda is extremely common.

Adults, however in many cases have no pallor of the skin and of the hair, yet all the other features of the syndrome are present. For this reason, a term such as *kwashiorkor* (the red boy or man), can hardly be applied to adult cases. Lastly the majority of cases in childhood show no crazy pavement dermatosis many show no looseness of the stools no psychosis has been described in this syndrome so these symptoms which at one time suggested a relationship to pellagra, do not appear to be the earliest or the most constant features of the syndrome.

A low temperature, cold extremities, and radiological evidence of moderate osteoporosis (twelve cases) were noted for the first time in this series.

### INVESTIGATIONS.

*Test Meal*—A gruel test meal in fifty-six adults gave achlorhydria in seventeen cases, four of whom were tested by histamine and two of whom still showed no secretion of acid.

*Radiological Examination of the Alimentary Tract*—This was performed in twenty three adults and in nine children. The appearance of defective



FIG. 1

FIG. 1—Pallor of the face brown hair and oedema in an adult. A normal control stands behind

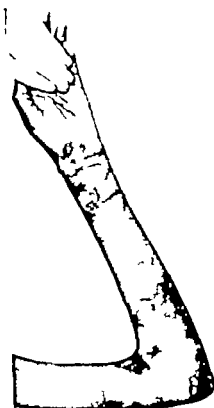


FIG. 4—Peeling of the dermatosis 7 days after giving nicotinic acid. The same case as in Fig. 1

FIG. 4

It is therefore considered that —

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A low temperature, cold extremities, and radiological evidence of moderate osteoporosis (twelve cases) were noted for the first time in this series.

### INVESTIGATIONS.

*Test Meal*—A gruel test meal in fifty-six adults gave achlorhydria in nineteen cases, four of whom were tested by histamine and two of whom still showed no secretion of acid.

*Radiological Examination of the Alimentary Tract*—This was performed in twenty three adults and in nine children. The appearance of deficiency



FIG. 3—Deficiency bowel pattern in the jejunum of a child of 2 years. Note the gross segmentation, the irregularities in the calibre and the coarse, poorly marked mucosal folds.



bowel pattern was reported in this syndrome by SCOTT BROWN and TROWELL (1944) and it is unnecessary to repeat it in any detail. Too little is known of the appearance of the normal African small intestine and although one of us (H. C. T.) was privileged to see the films from some 200 African soldiers (by the kindness of the military authorities) we do not feel that we have confirmed altogether the somewhat strict criteria of the normal small intestine, as set forth by ROSS GOLDEN (1941). We are inclined to accept only segmentation gross irregularity coarseness or loss of the mucosal folds in the small intestine as evidence of abnormality. ROSS GOLDEN believes that the small intestine should be even in calibre and have a fine mucosal pattern, if this is so then nineteen adults showed frank segmentation and five had an abnormal mucosal pattern none was normal. In children even more difficulty has been encountered by us in defining the criteria of normality, seven out of nine cases showed frank segmentation (Fig. 3) beyond that we will not go.

## INFECTIONS.

Infection.	Non fatal Cases (119)	Fatal Cases (23)	Percentage, compared with Single Examination of Routine Medical Admissions.
<i>Ascaris lumbricoides</i>	8 (6%)	1	0
<i>Necator americanus</i>	29 (57%)	4	46
Heavy infection			
Light infection	40	8	
<i>Taenia saginata</i>	16 (13%)	4	4
<i>Bilharzia mansoni</i>	1 (1%)	0	0.4
<i>Giardia intestinalis</i>	15 (12%)	5	0.1
<i>Chilomastix mesnili</i>	39 (32%)	8	0.1
<i>Trichomonas hominis</i>	0 (24%)	0	0.2
<i>Entamoeba histolytica</i>	9 (7%)	1	0.3

It is not considered that there was any significant increase of helminthic infections in the cases under review and it is very doubtful if these infections play anything but a minor part in the production of this syndrome. All protozoal infections appear to be more common in this syndrome than in the ordinary patients. This is especially true of the flagellate infections all of which appear to have been more common. In this connection it is interesting to recall that the only recorded case that has been traced in the British literature of similar abnormalities in the radiological appearances of the small intestine was reported by O'DONOVAN, MCGARTH and BOLAND (1942) in a patient in Ireland who had a *Giardia* infection.



The exhibition of mepacrine (atebrin) to our patients in the daily dosage of 0.3 grammes, although considered by KYZER (1941) and others to be almost specific in the treatment of flagellate infections, was only followed by slight improvement in all cases. The frequent attacks of loose stools occasioned considerable difficulty. At first the main concern was lest amoebiasis was being missed as cases had frequently been diagnosed as such in the past. Sigmoidoscopy was performed some sixteen times and in this way some four cases of amoebiasis, not previously diagnosed, were detected.

#### UNDIGESTED REMNANTS OF FOOD IN STOOLA

	Adults.		Children.	
	Non-fatal (119)	Fatal (5)	Non-fatal (46)	Fatal (10)
Starch	56	9	26	8
Meat -	33	6	15	6
Fat	h		6	8

#### THE BLOOD

Blood counts were performed on the peripheral blood of 144 adults (twenty five fatal, 119 recovered) and on fifty six children (ten fatal forty-six recovered).

Red Cells (millions)		Haemoglobin (grammes)		M.C.V. ( $\mu$ )		M.C.H.C. (%)	
Mean	Range	Mean	Range	Mean	Range	Mean	Range
Adults	119 recovered cases.						
2.2	1.0-3.2	6	2.5-12	11.9	71-124	29.8	19.2-39.4
Adults	5 fatal cases						
2.10	1.1-2.7	6.7	4.2-11.1	119-9	N. 150	29.8	24.5-37.9
Children	46 recovered cases						
3.19	0.67-4.83	6	3-14.1	116.5	84-135	27.6	4.4-37.4
Children	10 fatal cases.						
2.74	1.1-4.05	9	4.5-11.1	1 case only		1 case only	
Adults	30 cases, no malarial parasites, no helminth etc.						
2.02	1.30-4.64	9.6	4.9-13.8	106.6	81-115	30.7	23.0-33.4
Children	10 cases no malarial parasites, no helminth etc.						
3.19	1.97-5.6	11.0	3.1-11.1	2 cases only		2 cases only	

The syndrome is therefore usually accompanied by macrocytic hypochromic anaemia, but the anaemia may at times be normocytic, and although usually hypochromic the anaemia is often orthochromic.

Examination of the sternal marrow in ninety-eight adults and in three children revealed a cellular marrow in which early red cell precursors and mitotic forms were common. The prevailing type of erythropoiesis is undoubtedly normoblastic. In a large number of cases abnormal red cell precursors are seen towards the very extreme tail of the marrow smears. In discussing this aspect of the cases in a previous communication (TROWELL, 1942) it was suggested that these cells should be called nutritional macrocytic anaemia megaloblasts. After further consideration I do not consider these cells should be called megaloblasts for the nuclear structure is coarse and is much nearer to that of the normoblasts (the red cell precursors of normal marrow).

#### THE PLASMA PROTEINS

The plasma proteins were estimated by Howes modification of the Kjeldahl method in eleven adults and in three children.

#### PLASMA PROTEINS.

Case	Date	Total	Albumin	Globulin	A/G ratio
Adults					
1	3.8.43	7.2	2.37	4.82	0.49/1
	3.10.43	7.58	3.02	4.56	0.66/1
2	"	7.01	2.66	4.40	0.59/1
3	3.11.43	6.76	1.82	4.94	0.37/1
	15.12.43	6.49	3.56	2.93	1.22/1
4		4.32	2.71	2.61	1.69/1
5		6.95	1.10	5.84	0.19/1
6		7.08	1.58	5.50	0.29/1
7		6.87	3.10	3.78	0.82/1
8		6.05	2.12	3.93	0.54/1
9		8.51	1.80	6.77	0.27/1
10	7.12.43	7.24	1.99	5.25	0.38/1
	29.2.44	5.89	2.68	3.24	0.81/1
11	10.12.43	4.88	1.71	3.17	0.54/1
	29.2.44	6.98	2.80	4.18	0.67/1
Children.					
1		5.68	1.24	4.44	0.83/1
2		4.12	1.73	2.38	0.73/1
3		5.38	2.18	3.19	0.68/1

TABLE I—SUMMARY OF CLINICAL FINDINGS

No.	Mths. ill	Age and sex	Weight (lb.)	Oedema	Stool daily	Undigested Food	X-ray Jejunum	Test meal	Skin and hair changes	Parasites few (f) or many (m.)	Plasma Proteins. Alb. Glob.	R.B.C. (m l. ions)	Hb (grams)	M.C.V.	M.C.H.C.
1	3	3 M	31	+++	3-6	Meat fat, starch	Ab-normal	?	+++	R.T.M. (f) Ancy. (f)	1.72 2.34	3.10	9.8	?	?
2	3	30 M	144	+++	5-6	Starch	?	No HCl	+	Ternia Ancy. (f) Giardia (m)	?	31	6.8	120	96
3	3	30 M	140		2-3	Starch	Ab-normal	HCl	+	Ancy. (f) Chilomastix (m)	?	10	7.5	107	24
4	3	45 M	118	+	4-7	Starch	?	?	+	Nd	?	3-60	10-	?	?
5	1	50 M	102	+	6-7	Starch	Ab-normal	No HCl	++	Chilomastix (m)	2.10 2.28	3.18	11.1	91	24
6	3	18 M	90	+	2-7	Meat, fat, starch	Ab-normal	No HCl	+++	Ancy. (f) Trichostrongylus (m)	1.06 1.14	8.9	7.2	84	23

TABLE II—SUMMARY OF POSTMORTEM FINDINGS

No.	Death to P.M. (hours)	Infections	Helminths	Alim. Tract	Kidneys	Liver	Heart	Marrow
1	18	Bro. periton.	None	Normal	Normal	Congestion	Brown atrop hy	Hypert.
2	8	Bro periton	1 Ternia 18 Ancy. L	Normal	Tubul. degen.	Fatty degen.	Normal	Hypert.
3	3	Bro. periton.	10 Ancy. L	Normal	Tubul. degen.	Fatty degen.	Normal	Hypert.
4	11	Lob. periton.	3 Ancy. L	Normal	Normal	Fatty degen.	Aortic syphilia	Normal
5	3	Nd	None	Codman's carcinoma	Tubul. hyper.	Fatty degen.	Normal	Hypert.
6		Nd	8 Ternia	Normal	Tubul. degen.	Fatty degen.	Severe atrophy	Normal

The mean plasma albumin was 2.05 per cent. and the range varied from 1.10 to 3.02 per cent. This is much lower than the mean of about 4.5 per cent. for normal European adults. The mean plasma globulin was 4.05 per cent. and range varied from 1.44 to 6.77 per cent. This is much higher than the mean of about 2.5 per cent. for normal Europeans. The albumin/globulin ratio is therefore reversed and was usually below unity.

#### DEATHS AND POSTMORTEMS

*Adults*—Twenty-five deaths, twenty-one postmortems. Seven cases had known gastro-intestinal disease and are barely discussed. Fourteen cases had almost no naked eye abnormalities in the gastro-intestinal tract and were studied more carefully. Of these fourteen cases only five had been adequately examined in most of the routine procedures and are specially reported.

*Children*—Ten deaths, two postmortems. None are reported in detail.

Details are given of two adult cases for in them the investigations were fairly adequate. They were under observation for a fair period of time. They appeared to contract no terminal infection.

#### Case 5 Male Ruanda Native of some 20 Years

*History*—Left Ruanda 3 months prior to admission to walk to Uganda. Serious food shortages occurred and a monotonous diet of cooked cassava and plantains was taken on the way. Severe twisting abdominal pain, loss of appetite, loose stools, limb pains and paraesthesiae for 1 month.

*State*—Vulnerable, thin, weight 102 lb. profound anorexia and vomiting limited the intake of food. Slight oedema of legs. Hair straight, soft and brownish. Skin widespread rough, dry, cracked dermatosis on the back, thighs and legs. Stools six to seven daily. Undigested starch present, many *Chilomastix mesnili* flagellates. Plasma proteins and blood count as given in the summary. X-ray report on the alimentary tract: "Jejunum showed coarsening of the mucosal folds and segmentation. Barium passed rapidly into the ileum which showed considerable irregularity in width. At 7 hours the bulk of the barium was still in the small intestine, the upper and middle portions showed gross segmentation. Ileum irregular in outline and in calibre. At 24 hours the barium was just entering the colon, which was abnormal in appearance. The colon emptied rapidly." Achlorhydria to gruel test meal.

*Autopsy*—Much wasting of the subcutaneous tissues, muscles, and internal organs. Heart, 4½ oz. brown atrophy. Kidneys, right, 3 oz. left, 3½ oz. otherwise normal to the naked eye. Sections showed some haemorrhages into the tubules, but no other change. Liver, 30 oz. sections showed marked congestion, some fatty degeneration, slight malarial pigmentation. Spleen, 4 oz., slight malarial pigmentation. Few adhesions around the descending and sigmoid colon in which the mucous membrane was studded with small protruding masses, which were tightly packed together. These were usually from 0.5 to 1.0 cm. in size and protruded about 0.3 to 0.5 cm. into the lumen of the bowel and were tightly packed like polypi. Very firm pressure was needed to dislodge these masses from the wall. On doing so

shallow ulceration was seen. No macroscopic or microscopic signs of inflammation accompanied the superficial ulceration of the mucosa. The adherent masses were composed of fibrin, bacteria, and yeasts. No diverticula. No evidence of recent amoebiasis or bilharzia. The few plastic bands of adhesive peritonitis did not appear to have caused any obstruction. No abnormality detected in the sections of the stomach, duodenum, jejunum, ileum, or pancreas. No helminths found at autopsy. No abnormality in the naked eye appearance or in the sections of the lungs, brain, thyroid, pituitary or suprarenals. Active red marrow in the vertebrae, skull, sternum, and most of the femur and in the head of the tibia.

#### Case 6 Male Ruanda Native of some 16 Years

*History*—Two months in a famine area, lived almost entirely on cassava. Developed loose stools, oedema, ascites, pale skin and hair.

*Radiological Examination of the Alimentary Tract*—"At the preliminary examination the colon was tightly distended with gas, so that no barium passed the pylorus at the end of 3 hours. Given an enema and pepsin and a second barium meal, stomach emptied very rapidly and the barium passed through the small intestine very rapidly so that in 2 hours it had reached the hepatic flexure after which transit became slower. The upper jejunum was consistently narrowed to a width of 1.5 cm., but was regular in outline with normal plicae. The ileum was irregular in its width and had coarse irregular mucosal folds. The colon was normal. Diffuse osteoporosis was seen in all the bones of the skull, vertebrae and limbs."

*Autopsy* (within 15 minutes of death).—Little subcutaneous fat. Pits wasted muscles. Small amount of oedema of the legs. 5 pints of ascitic fluid. Stomach, poorly marked rugae, but the latter appeared normal in the small intestine. Colon, slight injection of the mucosa, which was covered by small amounts of purulent mucus, but there was no ulceration. No abnormality was detected in the sections of the stomach (fundus, body and pylorus), jejunum, mid-small intestines, ileum or caecum. The transverse and descending colon showed scanty polymorphonuclear infiltration of the mucosa but no ulceration. Liver 27½ oz., yellow and fatty cut surface. Sections showed much fat degeneration and some cloudy swelling with considerable deposits of haemosiderin pigment. Heart, small, 4 oz., brownish yellow muscle. Sections showed moderately scattered pigmentation, and brown atrophy. Kidneys left, 3 oz., right, 2½ oz. sections showed some congestion and cloudy swelling of the tubular epithelium. Pancreas appeared to be small, section appeared normal. Two small *Taenia saginata* (each about 2 feet long) and five hookworms.

#### MALNUTRITION SECONDARY TO CHRONIC GASTRO-INTESTINAL DISEASE

A group of seven cases showed all the signs described in this syndrome but in addition they had chronic intestinal disease. Apart from the latter, on other point of distinction could be detected. Only one representative case is described.

*Case 1 Male age 16 Years Bilharziasis Diarrhoea, 1 month. Advanced clinical signs yellow hair pale skin achlorhydria to histamine marked radiological changes in the intestine the stool contained no inflammatory cells until shortly before death when Schistosoma mansoni ova were found At autopsy no abnormality could be detected with the naked eye in the colon but sections of the pelvic colon revealed small collections of S. mansoni ova and slight surrounding inflammation. The liver revealed marked fatty degeneration, but no fibrosis and no Schistosoma ova.*

#### RESPONSE TO TREATMENT

Cases were at first given the usual hospital diet which consisted of the following average daily ration 6 lb of plantains 1 oz of meat, 1 lb of potatoes 1 oz. of beans This is approximately equivalent to calories 1870 protein 33.5 grammes fat 2.0 grammes, calcium 0.5 gramme iron 8 mg., vitamin A 2,500 international units, vitamin B<sub>1</sub> 1.8 mg riboflavin 1.1 mg nicotinic acid 1.5 mg ascorbic acid 300 mg On this basic diet progress was slow in every respect.

The effect of certain of the pure vitamins was studied on cases who received the basic diet. These cases were under observation for at least 10 days prior to the administration of the pure vitamin Aneurin (vitamin B<sub>1</sub>) was given to thirty three adults (twenty-eight orally five parenterally) in a dosage of 5 to 10 mg daily and to twenty children (ten orally ten parenterally) in a dosage of 1.5 to 5 mg daily In twenty-nine adults and fifteen children definite improvement was reported by the patients usually within 5 days. Improvement was largely subjective and consisted in a marked increase of appetite, an increased sense of well-being and in a slow decrease in the paraesthesiae in the limbs No change was noted in the oedema.

Nicotinic acid was given to nineteen adults (eighteen orally one parenterally as the nicotinic acid amide) in a dosage of 150 to 600 mg daily and to seventeen children (fifteen orally two parenterally) in a dosage of 30 to 100 mg daily In fourteen adults and in fourteen children improvement was observed usually within 7 days (Fig 4) This consisted in a peeling of the dermatosis The effect, if any on the diarrhoea, was not demonstrated. It proved impossible to assess which constituents of the diet corrected the gastro-intestinal defect which seemed to respond only extremely slowly to the combined action of the basic diet, supplemented by liver milk, aneurin and nicotinic acid.

Liver injections of crude liver extract were given to forty five non fatal patients twenty three of whom were adults and twenty two children. A satisfactory increase of red cells and haemoglobin, together with a reticulocyte crisis, occurred in almost all the cases in which macrocytic anaemia was present, and even in a few cases in which normocytic anaemia was present. (Blood counts were performed every 2nd day) Ferrous sulphate was also given

present in this syndrome have been produced by RAO (1941) in young monkeys fed on milled rice with supplementary foods in only small amounts. They developed diarrhoea and passed undigested particles of food the hair became coarse and sparse, the skin showed a scaly desquamation, oedema developed. At autopsy fatty degeneration of the liver was noted, as well as slight changes in the skin and nervous system and advanced degenerative changes in the gastro-intestinal tract. The syndrome was always fatal and advanced cases did not recover even when the diet was richly supplemented by all the known vitamins. Monkeys sat huddled up in a listless attitude, a description reminiscent of the enfeebled immigrant labourers, squatting hunched on the ground for hours on end, almost too weak to rise.

### CONCLUSIONS.

Is this a clear clinical syndrome? It is the opinion of many workers in the tropics that it is a distinct and separate syndrome and that it cannot be regarded as pellagra beriberi or nutritional oedema. No essential difference has been demonstrated between the syndrome as it occurs in children and in adults, apart from the rarity of gross oedema and much pallor of the skin and of the hair in adults. It follows that any term such as "kwashiorkor" (the red boy in the Ga language of the Gold Coast) is an accurate term for the disease as seen in African children but it can hardly be regarded as a fortunate term for the disease as seen in adults, or for members of other races. This redness is probably peculiar to the dark skin of the African this may explain why the syndrome has seldom been seen in Asia.

It is possible that if this condition is found to occur as a result of the war in Europe that our knowledge will rapidly increase. Meanwhile it may be termed perhaps "malnutrition" or "chronic starvation" although it does not correspond closely to accepted descriptions of starved people who usually recover rapidly when food is given. Not so this syndrome appetite is poor, food is poorly digested, absorbed and elaborated. It is for this reason that I suggested the term "malignant malnutrition" (TROWELL, 1944) to some of my colleagues in South Africa and found that on the whole the term commended itself to those who were actively investigating this disease.

### SUMMARY

1. Diets deficient in calories, protein and the vitamin B complex and other constituents produce a common syndrome of malnutrition in African adults and children.

2. The syndrome manifests itself as a failure of growth, or loss of weight, oedema, a gastro-intestinal defect, changes in the skin and hair anaemia, a fatty liver and slight neurological signs.

3 Detailed observations are offered of the failure to digest food, of the deficiency bowel pattern, of the lowered plasma albumin and the raised plasma globulin, and of two autopsies.

4 This syndrome appeared to be distinct from pellagra and nutritional oedema, it is very resistant to any improvement in the diet and to supplements of all the common vitamins and the disease has a high mortality

5 No satisfactory name has been proposed for this syndrome. Although kwashiorkor holds pride of place, as a description of the red-haired African babies, it has been proposed that it should be called 'malignant malnutrition' (TROWELL, 1944)

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## GROWTH OF PROTOZOA IN TISSUE CULTURE.

### 1 *PLASMODIUM GALLINACEUM* EXOERYTHROCYTIC FORMS

BY

FRANK HAWKING \*

*From the National Institute for Medical Research London, N W 3*

This paper describes attempts to cultivate *P. gallinaceum* *in vitro*. At first the endeavour was made to grow the asexual forms which are found in erythrocytes but the attempt was unsuccessful. Then tissue cultures were made of cells containing the exoerythrocytic stages, and by this technique vigorous growth of the parasites was obtained.

#### ATTEMPTS TO CULTIVATE ENDOERYTHROCYTIC FORMS

The first important attempt to grow malaria parasites *in vitro* was made by BASS and JOHNS (1912) their attempt was followed by many others. The literature has been reviewed by TRAGER (1941). In brief it has been possible by simple means to promote the growth of small trophozoites up to the stage of schizogony but evidence for the development *in vitro* of a second generation of parasites is mostly unconvincing. TRAGER (1941, 1943) working with *P. lophurae* introduced a number of technical improvements and succeeded in obtaining exflagellation of the male gametocyte after culture *in vitro* for 16 days. The writer tried to cultivate the endoerythrocytic forms of *P. gallinaceum* using a method similar to that of TRAGER including the salt solution which he recommends. Extract of red blood cells was prepared by defibrinating and centrifuging blood the serum was removed and the cells were haemolysed by freezing with solid carbon dioxide and thawing they were extracted with two volumes of Trager's solution K,† and the fluid was separated from the cellular debris by centrifuging. Infected blood cells were obtained from the heart of a suitable chick, and the blood was added to the culture medium in small Erlenmeyer flasks. The standard stock medium consisted of one part fowl serum, one part extract of red blood corpuscles and two parts Trager's solution K containing glutathione 0.2 per cent.

\* Grateful acknowledgments are due to Miss H. B. FELL, D.Sc. Dr W. JACOBSON and Dr F. JACOBY for instruction in the techniques of tissue culture to Miss I. M. TONKIN B.Sc., for the supply of infected chickens to Mr W. J. ELFORD Ph.D. Mr A. T. FULLER, Ph.D., Dr A. NEUBERGER and Professor C. REMINGTON for the preparation of tissue extracts and other assistance to Mr F. V. WELSH, F.R.S.L.S., for the photography and to Miss R. J. BRIDSON and Miss V. D. MAXHAM for technical assistance.

† NaCl 3.039 grammes, KCl 4.100 grammes,  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  0.690 gramme,  $\text{KH}_2\text{PO}_4$  2.613 grammes,  $\text{NaHCO}_3$  0.168 gramme,  $\text{CaCl}_2$  0.166 gramme,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.370 gramme,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  0.407 gramme, d. glucose 2.377 grammes, water to 1,000 c.c.

The rest of the technique was as described by TRAGER. All cultures were incubated at 37° C. incubation at 41° C. yielded poorer results. Growth of the parasites was studied by making films of the cells sucked off the floor of the flask and staining with Giemsa. Counts were made of the erythrocytes present in the whole medium and of the percentage of cells containing "normal" parasites in the smears. The arbitrary criteria of "normal" parasites was the presence of easily distinguishable chromatin. In some cases, conclusions were checked by inoculation of the culture into chickens, but usually the morphological appearance of the parasites was sufficient alone to show that growth was not satisfactory. In some cases a search was made for exflagellating male gametocytes as described by TRAGER. dark ground illumination was found an excellent method for demonstrating these. However some difficulty was experienced, because room temperature in England in war-time was often below the minimum temperature (217° C.) required for this phenomenon. Exflagellation was often observed in cultures which had been maintained for 3 days, but it was not observed later than this time. The effect of renewing the medium at intervals of 1 or 2 days was examined on a few occasions without observing much benefit to the parasites, but no great attention was paid to this aspect. The concentration of erythrocytes was usually only about 2,000 to 3,000 per c.mm. and the concentration of parasitized cells about 200 to 500 per c.mm. The volume of medium was so large in comparison with that of the cells, that it was considered that spoiling of the medium by the parasites was not likely to occur within the first 2 to 3 days, and that if growth failed to occur (as it usually did) failure must be due to initial unsuitability of the medium rather than to its spoiling by the parasites.

In the best flasks the percentage of parasitized cells (the parasites being judged by the criterion of whether chromatin was distinguishable) was as high on the 4th day as it was at the beginning. In one experiment the count on the 3rd day was over 900 per cent. of that at the start and there were many small forms present. but there are many possibilities of technical error in making these counts, and too much reliance should not be placed on them. A more representative example of a good experiment would be one in which the count of parasitized cells (compared with the count at the start) was 2nd day 80 per cent., 3rd day 63 per cent., 4th day 58 per cent. Cells were usually degenerate by the 4th day. In some experiments, the cultures were tested by injection into chickens. Chickens were infected after cultivation for 4 days and in one case after cultivation for 5 days. but the length of the incubation period suggested that less than 1 per cent. of the original number of parasites had survived. During the cultures there was a general tendency for the plasmodia to increase in size and in number of pieces of chromatin, so that the proportion of parasites with several nuclei rose. In some experiments tiny mononuclear forms were found in erythrocytes on the 3rd and 4th days which suggested that a second generation of plasmodia had been formed *in vitro* but on the whole achiogony and successful invasion of new erythrocytes seemed to be rarely accomplished. The gametocytes grew in size and survived better than the other types of plasmodia, and on the 4th and 5th days they were often the only forms which were not degenerate.

The strikingly favourable effect of red cell extract upon the growth and survival of the parasites, described by TRAGER, was confirmed. A similar beneficial effect was observed with glutathione. The optimum concentration of glutathione appeared to be about 0.1 to 0.2 per cent. concentrations greater than this were definitely harmful, while concentrations less than 0.1 per cent. were less actively beneficial. Cysteine could not replace glutathione. An attempt was made to ascertain the active principles in the red cell extract. Extract of red blood cells contains much glutathione but a mixture of extract and glutathione promoted survival of the parasites better than either component alone, so apparently the effect of the extract is due to other constituents besides the glutathione. Extract alone or extract plus serum was not a good medium for survival. Haemin had no action. An extract of rat erythrocytes seemed to be beneficial although probably less beneficial than chicken cells, so the action is not species specific. An extract of the cells dialysed through a collodion membrane promoted survival, so some of the active constituents have small molecules. Heating the extract to 75° C. for 10 minutes made it definitely toxic, and so did attempts to produce a protein free extract by means of precipitation with metaphosphoric acid and subsequent neutralization. These operations were kindly performed by Professor C. RIMINGTON. (It must be remembered that

these conclusions are based on qualitative impressions rather than on reliable quantitative measurements.) The very concentrated red blood cell extract described by TRAGER (1943) was tried, but it was difficult to prepare and seemed less beneficial than the extract made as described above.

The effect of various mixtures of oxygen and carbon dioxide was tried, but results were variable and inconsistent. Measurements of pH (carried out at 18° C. with a glass electrode allowing only a very short time for CO<sub>2</sub> to escape) similarly failed to permit any clear conclusion—relatively good survival occurred at pH levels ranging from 7.0 to 7.6 but in other experiments survival was poor within these limits. In some experiments the cultures became slightly acid during 4 days incubation, in others they became slightly more alkaline. Survival of the parasites seemed to be somewhat improved by rocking the flasks, by nicotinamide (0.0002 to 0.0002 per cent.) by pyridoxin (0.01 to 0.001 per cent.) by riboflavin (0.001 to 0.0004 per cent.) by p-aminobenzoic acid (0.002 per cent.) and by liver extract containing biotin. The effect of pantothenate (recommended by TRAGER, 1943) was studied in four experiments—concentrations of 0.001 to 0.0001 per cent. appeared to be beneficial but the effect was not great. Addition of fresh erythrocytes after 1 or 2 days appeared beneficial—but in experiments in which partial renewal of the fluid medium was undertaken, the parasites survived no better than the controls and often they survived less well. Survival of the parasites seemed to be impaired by heparin (0.005 per cent.) by glycerol (0.2 per cent.) by 0.3 or 0.4 per cent. glucose, by ascorbic acid (0.2 to 0.025 per cent. 0.0025 per cent. had no effect) or by altering the ratio of sodium and potassium from that recommended by TRAGER. No influence on the survival of the parasites was observed in the case of inositol (0.01 to 0.001 per cent.) of thiamin (0.05 to 0.0001 per cent.) of casein hydrolysate (0.001 per cent.) or of chick embryo extract. If the serum and red cell extract were prepared from a young chick instead of from an adult fowl the survival of the parasites was not materially improved.

It was concluded that apart from the beneficial effects of red blood cell extract and glutathione the other agents investigated caused little definite improvement in the survival of the parasites, and that no substantial multiplication of the parasites could be achieved. However the parasites can be maintained at 37° C. in fairly good condition for at least 2 days, which would be long enough for chemotherapeutic experiments *in vitro* analogous to those of YORKE and his colleagues with trypanosomes (YORKE and MURCATROYD 1930). In a single experiment carried out along these lines the minimal plasmodicidal concentration of quinine hydrochloride seemed to be in the region of 40 mg. per 100 c.c. after exposure for 24 hours, and more than 8 mg. per 100 c.c. after 48 hours—the minimum plasmodicidal concentration of mepacrine methane sulphonate seemed to be in the region of 1.2 mg. per 100 c.c. after 24 hours exposure and of 0.24 mg. per 100 c.c. after 48 hours.

*Attempts to infect chick embryos*—In collaboration with Miss I. M. TONEIN attempts were also made to infect developing eggs which had been incubated for 8 to 13 days. Blood containing trophozoites of *P. gallinaceum* was injected in a variety of ways. Altogether sixty nine eggs were used. Most of these died in the shell but fourteen of the chicks hatched out—all these showed no parasites in the blood at birth, but three of the chicks developed parasitaemia after 10, 14 and 14 days respectively. These results are similar to those reported by WOLFSON (1940) and by STAUBER and VAN DYKE (1945). It is concluded that chick embryos can be infected, but that they are probably less susceptible than the hatched chicks are. The resulting disease offered no particular advantages for our experiments.

#### TISSUE CULTURE OF EXOERYTHROCYTIC FORMS.

Since such poor results had been obtained during attempts to cultivate the endoerythrocytic stages of *P. gallinaceum* it was decided to try whether

tissue cultures made from organs containing exoerythrocytic forms might not be more successful. A description of these forms was given by JAMES and TATE (1938). The initial results of the present experiments have been described in a preliminary note (HAWKING 1944) in which a review of the previous literature was given.

GAVRILOV BORISOFF and LAURENCE (1938) made numerous experiments in which tissue from fowls infected with *P. gallinaceum* was cultured *in vitro*. Malpian parasites were never found histologically in these cultures but on two occasions material, cultured for 7 and 10 days respectively produced infections when inoculated into other fowls. Other experiments with inoculation of cultured tissue yielded no infections. HITCHCOCK and WALLMAN (1939) were primarily interested in attempts to determine whether exoerythrocytic forms were truly a stage in life-history of plasmodia or whether they were really a separate species of parasite altogether. They describe a single experiment in which tissue was taken from a canary infected with *P. cathemerianum* and cultivated *in vitro*. After 8 days material from the cultures was inoculated into fresh canaries and produced typical infection with *P. cathemerianum*. Material taken at a later date was not effective in producing infection. Exoerythrocytic forms were seen in smears made from one culture on the 15th day. No further investigation has been reported by these workers.

#### TECHNIQUE.

The chicks used in these experiments were usually 8 to 10 days old at the time of inoculation. Mosquitoes (*Aedes aegypti*) infected with *P. gallinaceum* were taken and the heads and thoraces were ground up in a small amount of heparinized plasma. The suspension was centrifuged for a short time at low speed to remove the grosser debris and then it was injected intravenously into the chicks. Usually one mosquito was allowed per chick but if more mosquitoes were available the incubation period was shorter. (This infection of chickens was carried out by Miss I. M. TONKIN.) About 1 day before the expected death of the chick, i.e., about the 8th to 9th day the chick was killed by chloroform. The spleen was removed, and minced with scissors. The fragments were suspended in Tyrode's solution and used as implants to insert into the flasks. If smears were made from these fragments and examined for parasites in the usual way exoerythrocytic forms could often be found but much searching was usually required to demonstrate them and they were quite rare compared with those subsequently found in the cultures. Implants of other organs (brain, liver or bone marrow) were prepared in the same way. In order to make cultures of the macrophages in the blood, the blood of the chicken was taken from the exposed heart with a pipette and placed in a cold paraffined tube. After centrifuging the plasma was removed and a drop of embryo extract was placed on the top layer (buffy coat) of the blood cells. When this top layer had clotted, it was removed, washed, and cut into small cubes to furnish implants.

The earlier cultures were grown in roller tubes the implants were embedded on a thin layer of plasma plus embryo extract, and the fluid phase was composed of twelve drops of Tyrode plus eight drops embryo extract.

Growth under these circumstances was quite good although the fibroblasts tended to outgrow the macrophages. The chief disadvantage was the difficulty of sampling. Portions of the culture could be sucked out with a fine pipette and smeared on a slide but since many of the cells were growing on the surface of the glass tube their removal was uncertain and they were usually much damaged in the process.

Accordingly a technique was adopted (with modifications) which was kindly suggested by Dr F. JACOB (1944). Carrel flasks were employed. Small glass slips were obtained by cutting up No. 1 cover slips. In most of the present work the slips measured about 1 cm. square but the exact dimensions are merely a matter of convenience. In a flask of 5.5 cm. diameter five (or six) of these slips can be used; in one of 3 cm. diameter there is room only for four slips. The correct number of slips is dropped into the flask and then by means of a platinum wire the pieces are arranged over the floor of the flask so that they lie flat without touching one another. Fowl plasma is run from a Pasteur pipette under each slip in order to glue it into position. If desired, the flask can be put into the incubator for 1 to 2 hours at this point to ensure firm clotting; alternatively the plasma may be mixed with embryo extract immediately before insertion. A small drop of plasma is then placed on top of each slip and the piece of tissue to be embedded is inserted into this drop. As much fluid as possible is sucked off with the pipette used for inserting the piece of tissue, so that the implant remains glued to the slip by only a thin coating of plasma. The flasks are left at room temperature, or 37° C. for  $\frac{1}{2}$  to 1 hour until the plasma is firmly clotted; then the fluid medium is inserted, and the mouth of the flask is closed by a rubber bung. The flask is incubated at 37° C.

The fluid medium usually contained 20 per cent. of serum obtained from young fowls. Blood was removed from the heart by a syringe and needle inserted down from the base of the neck; it was defibrinated by shaking with glass beads, and centrifuged; usually the serum contained a little haemoglobin. Experiment showed that growth was not much more vigorous if the proportion of the serum was increased as high as 50 per cent. and that it was not much worse if the serum was only 10 per cent.; fair growth occurred even in 6 per cent. serum. Accordingly 20 per cent. was chosen as an economical level. The medium also contained 1 to 2 c.c. embryo extract to 30 c.c. of medium (embryo extract was prepared by mincing 8 to 10 day old embryos in Tyrode, 3 c.c. Tyrode per embryo and centrifuging). The embryo extract was not essential but its presence caused more rapid growth; higher proportions were avoided, since it was desired to encourage the growth of macrophages and discourage that of fibroblasts. The medium contained 0.05 per cent. phenol red to indicate the reaction. The initial reaction of the Tyrode solution used was often about pH 8.2 and during most of this work no steps were taken to modify it. In later work expired air was blown through the

Tyrode to reduce the pH to about 7.6 to 7.8 before adding the other constituents when the medium in the flasks was changed this procedure was omitted as the contents of the flasks were already somewhat acid. Finally the medium contained penicillin approximately 3 units per c.c. The stability of the penicillin present in these cultures was kindly measured by Dr A. T. FULLER in a culture which initially contained 15 units of penicillin per c.c., the concentration of active penicillin was 1 unit after 1 day 0.4 unit after 2 days, 0.3 unit after 3 days and 0.05 unit after 6 days. The penicillin did not interfere with the growth of parasites while helping to prevent the growth of bacteria. Occasionally heavy bacterial contamination occurred which the penicillin was unable to prevent at other times, however a few bacteria were seen in the cultures but they disappeared later presumably on account of the penicillin. The penicillin was dissolved in sterile Tyrode's solution in a concentration of 100 units per c.c. and added to the medium in an appropriate dilution. The phenol red was similarly dissolved in concentrated solution in Ringer or Tyrode, sterilized by autoclaving and added as required. Accordingly in a typical experiment the composition of the medium was serum 20 per cent. embryo extract 7 per cent. or less phenol red 0.05 per cent. penicillin 5 units per c.c., Tyrode 73 per cent. The medium was changed every 5 (or 4) days perhaps a shorter interval would often have promoted better growth, but the 5-day period was more economical of labour.

Examination of the general growth of the culture was made by inverting the flask and inspecting the cells with the low power of the microscope. For more precise examination the flask was opened and one of the glass slips was drawn into the neck of the flask by means of a platinum wire hook. The slip could then be picked up in fine forceps. The slip was rinsed for a few seconds in Ringer's solution (this washing was later omitted) and then dropped into methyl alcohol (1 hour) for fixation. In the later work Schaudinn was used. Most of the slips were stained for 1 hour in 10 per cent. Giemsa buffered to pH 7.2, washed, dried, and mounted in a neutral material (Gurr's neutral mountant). In some cases the slips were stained with Delafield's haematoxylin, or Dobeil's iron haematoxylin and dehydrated by passing through alcohol, xylol, etc. Some cultures were examined in the living condition by placing the slip bearing the culture cells upwards on an ordinary slide, moistening with Tyrode and using a water immersion objective. Identification of the parasites was difficult or impossible.

Probably this technique could be modified in many ways and good (or better) results could be obtained.

Some cultures were also made as hanging drops in a medium of equal parts plasma and embryo extract. Vigorous growth of cells could not be maintained for more than 2 to 3 days without changing the medium or transplanting, which were laborious procedures often resulting in infection. Growth of parasites was seldom obtained, and examination as a stained preparation was difficult. Accordingly little use was made of this method of cultivation.

## EXPERIMENTAL RESULTS

When cultures are made from the spleen by the technique described, migration of rounded and of elongated cells begins during the first 24 hours. During subsequent days these cells migrate further afield and in vigorous cultures they eventually spread over most of the floor of the flask in a rather diffuse arrangement. The predominant cell is one with circular or somewhat stellate shape with a round or oval nucleus but there are many cells (apparently closely similar) with numerous tapering processes no attempt has been made to venture on the finer classification of cells in culture and both these types are included under the designation of macrophage. Other cells are narrow spindle shaped cells with an elongated nucleus and these have been considered to be fibroblasts they are most prominent in the early stages of the culture and after the first week or two they become rare. Other types of cells are seen in the 1st and 2nd days but they soon die out. After the first few days one or more clear circular areas appear in the sheet of cells forming the colony. These areas are surrounded by fibroblasts arranged circumferentially several cells deep (Fig 25). Inside these areas the plasma of the original clot appears to have been removed, and macrophages (spread very thin) grow over the glass surface thus exposed this is a very favourable site for examination of parasites. Parasites may occur in all parts of the colony but they are usually most numerous in the thicker layers round the clear areas slightly less numerous in the clear areas and relatively rare in the peripheral parts of the culture. In many cultures after the first week or two the cells often contain numerous globules or fine vacuoles in the cytoplasm, presumably an indication that the conditions of culture are not altogether satisfactory. In older cultures (about 4 weeks) large multinuclear cells containing twenty or more nuclei often develop. Such cells less commonly contain parasites. After 1 or 2 months the cells gradually become more unhealthy in every way and their numbers diminish.

The number of parasites found in the early stages depends upon the number of exoerythrocytic forms present in the original implant. Usually it has required long search to demonstrate exoerythrocytic forms in smears made from the spleen used to provide implants, but parasites have been found fairly easily in the cultures after 2 or 4 days incubation. The development of the parasites cannot be followed by continuous observation, but its course may be reconstructed from a study of the different forms seen, which are reproduced in the illustrations. The description given is based upon preparations stained with Giemsa there has not yet been the opportunity for study by finer cytological methods. The earliest form is a small rounded parasite with a single piece of chromatin the apparent size depends upon the age of this form and also upon the extent of its flattening (Fig 1). The chromatin divides into two and further divisions take place until eventually schizonts are formed



containing very many (more than fifty) pieces of chromatin as shown in Figs. 8 and 9. Forms are sometimes seen in which there are two to five clear blue areas of cytoplasm with the pieces of chromatin arranged as a single layer round them (Fig. 10-11). This has been interpreted by JAMES and TATE as the first stage of segmentation or schizogony and the subdivisions of the schizont have been called cytomeres. Their interpretation is probably correct, although in the absence of continuous observation it cannot be proved. It must be noted, however, that sometimes the pieces of chromatin seen in these forms appear less numerous than in many other schizonts which do not show this arrangement. The present technique (in which trauma during fixation is practically absent) clearly shows that the above forms are always intracellular. Exoerythrocytic schizonts found in smears *outside* cells have almost certainly suffered mechanical displacement during preparation of the smear.

At a somewhat later stage of development, however, large numbers of elongated merozoites (Fig. 14) may be found living mostly *outside* the cell. These forms seem not to have been fully described before. They measure about  $2.4 \times 0.8 \mu$ . One end is pointed while the other end is often rounded. The chromatin is round or oval, and often it seems to project beyond the edge of the cytoplasm. A dark outer ring of chromatin and a less dark centre can often be distinguished. Often there is a granule in the cytoplasm which is mostly (but not always) at the blunt end of the parasite. This granule is presumably the same as the volutin granules seen in the intracellular forms (see below). It often occurs in cultures which appear healthy. On the other hand, in other vigorous cultures it is rarely seen. These merozoites are obviously formed by the breaking up of the large schizonts with numerous pieces of chromatin, but the exact stages of this process are not altogether clear. From a study of preparations (such as Figs. 12, 13, 22, 27 and 28) in which the schizont appears to be in the phase of disintegration, or to have just disintegrated, it is considered that when the merozoites are first liberated, they have a rounded form and that they become elongated as they pass out of the host cell. But a few forms have been encountered in which the merozoites seemed to be elongated before they broke away from one another. The outlines of the cytoplasm are often difficult to trace and at present no dogmatic statement can be made on the matter. In these cultures the host cell is *not* completely filled by the mass of merozoites, as reported by JAMES and TATE concerning

FIGS. 1 to 6.—Parasites with one to nine pieces of chromatin.

FIG. 7.—Parasites with granules and vacuoles, possibly slightly degenerate.

FIGS. 8 to 9.—Larger schizonts.

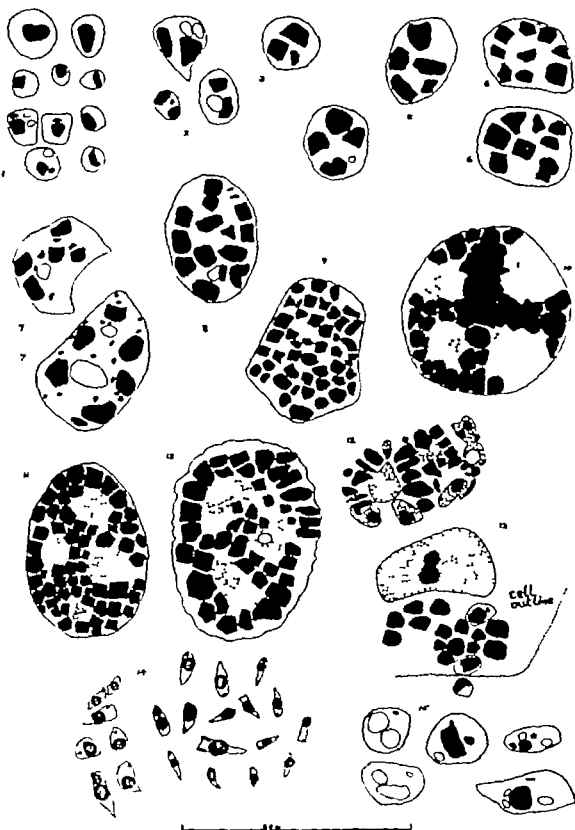
FIGS. 10 to 11.—Schizonts with the chromatin arranged round clear areas of cytoplasm (formation of cytomeres).

FIG. 12.—A schizont breaking up into merozoites.

FIG. 13.—Ditto, the nucleus and outline of the host cell are shown.

FIG. 14.—Two groups of merozoites.

FIG. 15.—Degenerating parasites with vacuoles and loss of chromatin in two isolates.



Illustrations 1 to 15 were drawn freehand by F. H. (magnification as shown by scale)  
 16 to 24 were traced by V. D. M. from photographs (magnification  $\times 470$  approx.)

parasites in the chick. It is not certain whether the host cell is destroyed when the merozoites are liberated or whether it survives. When the merozoites emerge from the host cell they enter adjacent cells and the cycle begins again. Apparently they remain within the immediate neighbourhood of their origin, for distribution of the parasites within the culture is very patchy—some areas contain no parasites, while in other small areas parasites are very numerous and multiple infections of the cells are common and intense. As many as twenty parasites in one cell are not infrequent.\* The duration of the cycle is not known. A typical culture contains parasites at most stages of development. However it may possibly be significant that to date clusters of merozoites have been found only in cultures more than 7 days old.

The cytoplasm of the earlier stages of the parasites often contains a small vacuole—it is not clear whether this is physiological or whether it indicates early pathological change. In some cultures the cytoplasm also contains one or more granules staining dark brown with Giemsa (Fig 7). These granules are often in close relation to the chromatin. (They are depicted also in Fig 6 of the paper by JAMES and TATE.) Again it is not clear whether they can occur in healthy parasites or whether they are pathological. Certainly they are most pronounced in parasites which are clearly degenerating as is shown by the presence of large vacuoles, and (in some cases) by the loss of chromatin (Fig 15). The degeneration of the parasites shown in this figure was probably due to the general degeneration of the host cell in which they lay.

The cells which contain parasites are mostly the rounded cells which have been identified as macrophages. Sometimes parasites also occur in elongated cells with long tapering processes which bear a superficial resemblance to fibroblasts (Fig 19). However Dr H. B. FELL kindly examined some of these preparations and considered that these cells were not fibroblasts but reticulo-endothelial cells.

The tissue most commonly employed as a source of these cultures has been spleen, since this is easily obtained and gives very satisfactory growth. Good cultures have also been obtained from bone marrow and from buffy coat of centrifuged blood but these tissues require more manipulation to obtain growth. Growth from the liver is somewhat less good than from spleen. Cultures from the brain have been unsuccessful in spite of the large number of exoerythrocytic forms which this organ contains—a fair growth of round cells and of cells with long processes takes place but no parasites survive. In a few experiments, cultures have been made from the heart and lungs, but parasites did not grow.

In Fig 2 of the preliminary note (HAWKING, 1944) a cluster of small forms was interpreted as due to the division of a schizont—it is more probable that they represented multiple infection of a cell, spread out when the smear was made.

FIG. 18.—Cell with small mononuclear parasites.

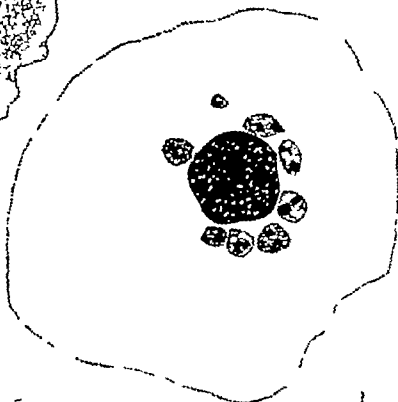
FIGS. 17 to 18.—Ditto with larger parasites.

FIG. 19.—Parasite in reticulo-endothelial cell.

*Duration of the cultures*—Living parasites have been demonstrated by inoculation of chickens in four different cultures after 89 days 82 days 80 days and 58 days respectively Termination of these cultures was due to bacterial



16



17

18



19



infection while changing the medium. Parasites have been demonstrated microscopically after 61 days culture—it should be remembered that one Camd flask cannot be sampled more than four or five times since the number of glass slips is then exhausted. In these cultures the fluid medium was changed at 5-day intervals so as to economize labour—more frequent attention might have prolonged growth. Dr F. JACOBY has kindly informed the writer that it is seldom possible to maintain cultures of fowl macrophages for more than three months—this fact would limit the duration of life which could be expected for any individual culture of *P. gallinaceum*. However if the parasites could be subinoculated into new cultures of uninfected spleen tissue (see below) there seems to be no reason why they should not be maintained *in vitro* indefinitely. In view of the great amount of care and labour required to be sure of maintaining cultures over a long period, further attention to this aspect of the question was postponed.

#### Environmental conditions

A few observations have been made on the effect of environmental conditions upon the growth of the parasites—probably the effect of most of these is exerted primarily on the cells and secondarily upon the parasites. Concentrations of serum greater than 10 per cent. (up to 50 per cent.) do not produce any marked improvement in growth—some growth will occur in concentrations of 6 per cent. or even 3 per cent. serum but it is less extensive than in 10 per cent. A trace of embryo extract improves growth, but it is not essential. The cultures can also be carried out in extracts of embryo liver or spleen in the absence of serum (apart from that contributed by the plasma used for embedding the implant)—in these circumstances the growth of fibroblasts is favoured as compared with that of macrophages. Parasites and cells will grow in atmospheres of 10 per cent. and of 40 per cent. oxygen, as readily as in air. Cells grow quite well in 100 per cent. oxygen—the position about parasites is not clear. When the flasks are filled with nitrogen (traces of oxygen present) growth of cells and parasites is very limited—under more completely anaerobic conditions as in a Fildes jar no growth of cells occurs. Penicillin in concentrations of 10 units per c.c. does not affect growth. Good cultures were obtained in the presence of glutathione 0.01 or 0.001 per cent., but no growth of cells took place in 0.1 per cent. glutathione. Red cell extract (see above) did not markedly influence growth. Since growth of cells and parasites is

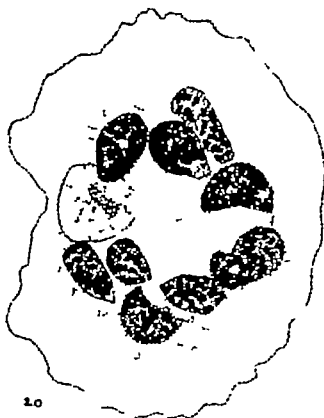
FIG. 20.—A large macrophage containing nine schizonts, culture from buffy coat 6 days.

FIG. 21.—Macrophage with two schizonts with peripheral arrangement of chromatin. Culture from buffy coat, 6 days.

FIG. 22.—Schizont with formation of cytomeres, host cell not shown.

FIG. 23.—Schizogony with the liberation of merozoites. The cells are macrophages. Culture from spleen, 24 days.

FIG. 24.—Merozoites formed after schizogony. Culture from spleen, 15 days.



already vigorous under standard conditions it would be more difficult to demonstrate stimulation of growth than it is to demonstrate its impairment.

### Transmission of parasites.

(a) *To chickens*—If the fluid from the flasks is centrifuged and the deposit examined, a variable number of (partially degenerate) macrophages is found together with parasites at various stages including merozoites. If this fluid or a scraping from the culture is injected into chickens intraperitoneally or subcutaneously infection occurs. There is a latent period of about 6 days, and then a few trophozoites begin to appear in the blood and to increase in number but before they have increased beyond a small figure e.g., 5 to 10 per cent. of red cells parasitized, the chicken dies from infection of the brain capillaries by exoerythrocytic forms. Accordingly the course of the infection resembles that produced by injecting sporozoites rather than that following injection of trophozoites.

(b) *To other tissue cultures*—As was pointed out above if the infection could be transmitted to clean cultures there is no theoretical reason why the parasites should not be passaged *in vitro* as long as is desired. Certain experiments were arranged so that the Carrel flasks contained one implant of infected spleen together with four implants from clean spleen which was maintained to see if parasites would pass from the cultures of the infected spleen to those of the clean spleen. Out of ten such flasks transmission occurred only in one after incubation for 25 days without rocking. Three similar flasks were set up again the flasks being placed on a rocking machine so the fluid oscillated slowly from side to side in all three flasks infection could be demonstrated in the clean cultures of cells after about 17 days however an attempt to pass the infection a second time was unsuccessful. In other experiments the surface of an infected culture was gently scraped with a spatula or pipette and the scrapings were applied to the surface of the clean cultures temporarily devoid of fluid this seemed to be the simplest and most certain method of transferring the infection, but here again a few attempts at second passages were unsuccessful. It is concluded that transmission of the infection to clean cultures is possible, but that it does not take place readily this agrees with the observation

FIG. 25.—Portion of the edge of a 7 days' culture from the spleen. On the left seen is part of the ring of fibroblasts surrounding the clear space (on right) in which macrophages are growing. ( $\times 85$ )

FIG. 26.—Four typical schizonts in a macrophage. Culture from buffy coat after 10 days. ( $\times 1250$ )

FIG. 27.—A schizont dividing into merozoites. Culture from buffy coat after 7 days. (Smear preparation.) ( $\times 1250$ )

FIG. 28.—A schizont breaking up into merozoites. From spleen after 6 days. ( $\times 1250$ )

FIG. 29.—Merozoites formed by the breaking up of a schizont. Culture from spleen 15 days. ( $\times 1250$ )





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FIG. 28.—A schizont breaking up into merozoites. From spleen after 6 days ( $\times 1250$ )

FIG. 29.—Merozoites formed by the breaking up of a schizont. Culture from spleen 15 days. ( $\times 1250$ )

described above that infection is often concentrated in one small part of the cell-colony while other parts remain free. Merozoites do not readily pass far from their point of origin.

(c) *To erythrocytes*—Several experiments were made in which erythrocytes plus red cell extract were added to the fluid medium in flasks containing vigorous cultures. The flasks were rocked. The erythrocytes remained in good condition for over 4 days, but no evidence of their invasion by parasites could be detected. However as was described above it is not possible at present to cultivate trophozoites *in vitro* and this absence of infection may be due to deficiencies in the methods of culture rather than to lack of power of the parasites to invade erythrocytes.

#### *Cultivation from cryptozoites and metacryptozoites*

The standard cultures described above were made from chickens infected intravenously with one infected mosquito per chick, and the tissue was removed from the bird when a period of 7 to 8 days had elapsed *i.e.* not long before the bird might be expected to die from the disease. At this stage the spleen is dark purple and greatly swollen, and exoerythrocytic forms can be demonstrated in it by careful search. smears of the brain contain many exoerythrocytic forms. An investigation was carried out to see how short the period between inoculation of the chick and taking tissue for culture might be made and growth of the parasites might still be obtained. The supernatant from a suspension of mosquitoes (mostly infected) was injected intravenously into chicks and the chicks were killed after a suitable interval which was made progressively shorter in each experiment. The spleen in chicks killed 4 days after inoculation was slightly enlarged in the others it was normal in size and pale. In smears made from the spleen of the chick killed 3 days after inoculation one schizont containing about 24 pieces of chromatin was found after long search in smears from the spleens of chicks killed after shorter intervals no parasites could be found microscopically. Cultures were made from the spleen in the usual way. Samples were removed at intervals mostly after 2 to 3 weeks for examination. In addition pooled fluid from the flasks was often injected intraperitoneally into chicks to demonstrate the presence or absence of parasites. The results in this series of experiments are summarized in the table. Infective parasites were found in cultures made from the spleen of a chicken which had received sporozoites only 1 hour earlier (The negative results obtained when the interval was only 15 minutes have no strong significance, and the investigation is being continued.) On the whole the longer the interval between injection of sporozoites and removal of tissue for culture, the easier it was to demonstrate parasites.

In these cultures from chickens killed at a short interval after inoculation the number of parasites is usually small, even after incubation for 2 weeks and the parasites are concentrated into a few foci as though each focus was

derived from a single cell. Morphologically the forms are identical with those described above. All types have been seen in these cultures but parasites

SUMMARY OF EXPERIMENTS IN WHICH TISSUE CULTURES WERE MADE FROM THE SPLEEN OF CHICKENS AT VARIOUS INTERVALS AFTER THEY HAD BEEN INOCULATED INTRAVENOUSLY WITH SPOOROZOITES OF *P. gallinaceum*.

Interval between Inoculation and Removal of Tissue for culture	Number of Mosquitoes Injected per Chick.	Number of Cell Colonies Examined.	Results.	
			Number of Colonies containing Parasites.	Infectivity of Fluid for Chick.
5 day		5	at 11th day	
4 days	40	6	1 4th day 1 6th 1 1st " 4th	
3 day	40	8	9th 1 12th 1 16th	
days	40	0	18th	
27 hours	40	6	1 18th	
24 hours	3-3	10	1 4th 2 7th 1 12th	+ 9th day
12 hours	100	4	1 8th	+ve 4th and 7th days
6 hours	110	7	1 5th	+ve 9th and 17th days
3½ hours	90	70	1 12th 4 1st	→ 9th day + 17th and 1st day
1 hour	70	1	1 16th	+ve 4th, 6th and 22nd days +ve 14th and 16th days
15 minutes	70	9	No parasites found	→ 4th to 22nd days (14 chicks)

with a single piece of chromatin often predominate. A limited number of sporozoites injected into a chick can produce a heavy infection throughout the whole bird in less than 9 days and even a single sporozoite multiplying at the

same rate would soon produce enough parasites to swamp the tiny volume occupied by one of these tissue cultures. Since this is far from occurring the rate of multiplication in these cultures is clearly much less than that which takes place *in vivo* under suitable conditions. The slow and scanty growth of parasites in these cultures under the present technical conditions, would constitute an obstacle in utilising this method to study the development of sporozoites of *P. gallinaceum* in avian tissues.

*Attempts to cultivate sporozoites*—Sporozoites suspended in serum or heparinized plasma were obtained and were added to cultures of clean spleen tissue. Some of the cultures became contaminated with bacteria but many remained sterile (penicillin present). No growth of parasites could be demonstrated in spite of various devices such as increasing or decreasing the oxygen pressure, modifying the pH using cultures from various parts of the embryo, or incubating clean spleen tissue with sporozoites before implantation. In view of the success obtained by injecting sporozoites into a chick and making cultures from the spleen 1 hour later, it seems probable that this failure to infect cultures of clean tissue by means of sporozoites was due to some error of technique rather than to any fundamental obstacle inherent in the parasite.

### DISCUSSION

The work described above seems to constitute the first satisfactory demonstration that malaria parasites can be cultivated *in vitro* for any considerable length of time but it must be admitted that this demonstration is restricted to the exoerythrocytic forms of *P. gallinaceum* and that satisfactory cultivation of the endoerythrocytic forms has not been achieved. The growth of any of these forms extracellularly although theoretically possible, seems likely to involve so many intimate problems of cell metabolism that its achievement may be relegated to the distant future. Even with exoerythrocytic forms multiplication of the parasites seems to be much slower in tissue culture than it is in the chicken otherwise the small volume of tissue present in these flasks would soon become overgrown by parasites, which does not happen.

The life-cycle of the exoerythrocytic forms which occur in these cultures conforms with that described by JAMES and TATE (1938) but the elongated merozoites are more conspicuous in tissue culture than in smears from the chick and the host cell is not so completely occupied by the parasite as seemed to be the case in their preparations. In the present cultures there was great variation in the appearance of schizonts but no evidence for two distinct types was obtained. The relation of the forms studied in this paper to the stages intermediate between sporozoite and trophozoite (HUFF'S cryptozoites and metacryptozoites DAVEY'S primary tissue phase) cannot be discussed satisfactorily in this article. Although cultures can be made from these early stages it may be noted that cultivation is less easy than it is with the forms found

about the 8th day after infection and that very heavy inoculations of sporozoites into the chicken are required.

It is hoped that this technique will prove helpful in elucidating some of the still obscure phases of the life history of malaria parasites. It permits examination of the effect of drugs upon one specific phase of the parasite (the exoerythrocytic phase); preliminary studies on this aspect have already been made by Miss I. M. TONKIN. Possibly the technique will assist a study of the biochemical requirements of malaria parasites, although it will generally be difficult to distinguish between effect upon the parasite and effect upon the cell in which it lies.

The present work has dealt only with *P. gallinaceum*. The single experiment reported by HEYER and WOLFSON (1939) indicates that *P. cathemerium* can be cultivated in the same way and work in this laboratory (carried out with the kind co-operation of Miss A. BISHOP, D.Sc.) has shown that the same is true of *P. relictum*. Presumably all species of malaria parasites in which a tissue phase can be demonstrated ought to grow under these conditions. The possibilities of the method, however, are not limited to the cultivation of plasmodia. Any intracellular parasite which lives inside a type of cell which can be grown *in vitro* ought to prove susceptible to cultivation by this technique. In a few preliminary experiments it has been found that *Trypanosoma evansi* will grow vigorously in cultures of rat embryo (HAWKING 1945), and that numerous forms of *Leishmania donovani* can be found in tissue cultures made from the spleen of infected hamsters. The results of this and other work will be described in later papers.

### SUMMARY

A technique is described for the cultivation of the exoerythrocytic form of *Plasmodium gallinaceum* in tissue cultures of chicken cells of the macrophage reticulo-endothelial type. Cultures may be made from the spleen, buffy coat, marrow and many other organs. Active multiplication of the parasites occurs. The developmental cycle of the parasites under these conditions conforms to the description given by JAMES and TAYLOR (1939).

Living parasites have been recovered from these cultures after 89 days. Growth occurs in the presence of penicillin, 10 units per c.c.

Chickens can be inoculated from the cultures. exoerythrocytic forms appear at an early stage of the resulting infection which thus resembles the infection produced by sporozoites rather than that produced by trophozoites. The infection can also be passed to cultures of clean chicken tissue but passage is difficult and uncertain. It has not been possible to infect erythrocytes from these cultures.

Cultures have been made from the spleen of a chicken inoculated intravenously with sporozoites 1 hour previously. It has not been possible to infect cultures of chicken cells by means of sporozoites.

Attempts were made to cultivate the endoerythrocytic forms of *P. galinaceum* using a modification of the technique described by TRAGER (1941). Some parasites survived for 5 days but no satisfactory multiplication could be obtained. Survival of the parasites was greatly improved by the presence of erythrocyte extract and of glutathione.

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# A RAPID METHOD FOR DETECTING THE SICKLE CELL TRAIT

BY

G. ROBINSON M.B., CH.B.,

*Senior Pathologist Medical Research Institute Accra, Gold Coast*

NEUDA and ROSEN† record the occurrence of rapid sickling of red corpuscles when a drop of blood from a case of sickle cell disease is mixed with a drop of ferment broth prepared by inoculating ordinary broth with 0.1 ml. of the filtrate from an emulsion of formed faeces in saline.

In an investigation of the obstetrical history of African women with the sickle cell trait, I have used broth cultures of various bacteria for rapid detection of the trait. Some bacteria behave better than others in this respect, and the one most frequently used by me has been *Pseudomonas fluorescens* Bact. cul. *Staph aureus* *B subtilis* have also been used.

It is obvious that the "ferment broth" of Neuda and Rosen is really a mixed culture of intestinal organisms.

It appears that the presence of living bacteria is necessary for the rapid production of sickling by this method. The following observations have been made —

- 1 The filtrates, after filtering through Seitz Disc EH/3 and Pasteur Chamberland Candle L3 were inactive.
- 2 Heat, 60° C for 1 hour renders the cultures inactive
- 3 Alcohol killed bacteria are inactive.
- 4 Suspensions, in saline or broth of bacteria from Agar cultures are active without further incubation.

## Method

A drop of a 24- or 48-hour-old culture previously tested for its activity against known sickling corpuscles is placed on a slide and a smaller drop of finger blood mixed with it, covered with a cover glass and examined under the microscope

In cases with the trait, or with active sickle cell anaemia, sickling of the corpuscles begins to show in from 1 to 5 minutes in most cases 70 to 90 per cent. of the corpuscles being sickled in 15 minutes.

\* I have to express my thanks to Dr W. M. HOWELLS, O.B.E., Acting Director Medical Services, Gold Coast Colony for permission to publish this note

† NEUDA, M. & ROSEN, M. S. (1945) *J. Lab. clin. Med.*, 30 (5), 456.

TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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VOL. XXXIX. No 4 FEBRUARY 1946

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LABORATORY MEETING

of the Society held at the

London School of Hygiene and Tropical Medicine,  
Keppel Street, London,

on

Thursday, 15th November, 1945, at 8 p.m.

C. M. WENTON C.M.O. C.B.E. M.B., B.S., B.Sc., F.R.S.  
President, in the Chair

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DEMONSTRATIONS

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London School of Hygiene and Tropical Medicine

Mrs B. Gilchrist

Laboratory experiments designed to study the factors which influence the female mosquito in choosing a place for egg-laying

Experiments were carried out to determine if when offered a choice, the female of *Culex molestus* would select a particular concentration of a simple salt solution in which to lay her eggs

Preliminary experiments with solutions of sodium chloride had shown that the female mosquito would readily oviposit in a concentration of 0.3 per cent. or less, but would seldom do so in concentrations above 0.9 per cent. In each experiment, therefore the mosquitoes were given the choice of distilled water and three concentrations of the same salt iso-osmotic with 0.3 per cent., 0.6 per cent. and 0.9 per cent. sodium chloride



The results of these experiments were shown in the demonstration. These indicate that the gravid female shows a preference for a solution having an osmotic pressure equal to that of 0.3 per cent. sodium chloride, irrespective of whether the salt is  $\text{NaCl}$ ,  $\text{KCl}$ ,  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{K}_2\text{SO}_4$ , or  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ . A similar result is obtained if iso-osmotic solutions of sucrose are offered.

It would seem, therefore, that the female is influenced in her choice either directly by the osmotic pressure of the solution or by some factor which varies with it as, for example, surface tension or vapour pressure.

Prof. P. A. Buxton

Film in colour showing tsetse breeding places and tsetse control in East Africa

The film, recently taken in Kenya, Uganda and Tanganyika, showed typical haunts of *Glossina morsitans* and *sergentorum*—air snapshots of clearance against *G. palpalis*—such methods of study as the use of bait cattle, and cloth screens. It also showed felling and clearing against tsetse, and a number of pictures of healthy cattle, villages and cultivation in areas formerly occupied by fly.

Dr. J. R. Buxvine

Demonstration of experiments on the formation of DDT crystals on a surface

The insecticide DDT tends, under certain conditions, to form super-saturated droplets (from drying solutions) or super-cooled droplets (from thermally dispersed aerosols). These droplets may collect on surfaces and remain liquid for weeks when undisturbed. But the contact of a paintbrush or an insect walking over the surface causes rapid crystallization.

An aerosol smoke from the insecticide, Gammexane, was found to deposit remarkable dendritic crystalline growths on a spider's thread.

Col. H. E. Shortt

1. Slides of *Plasmodium knowlesi* thin smear and saponized and centrifuged: the latter to show parasites removed from the red cells.

In the slide treated with saponin the erythrocytes have been removed and the parasites are lying free. By centrifuging, massive quantities of the latter can be prepared. These parasites are still viable and will produce infections if inoculated into a monkey.

2. Slides of *Toxoplasma* sp.

A natural infection in a guinea pig from Malaya. Smear made from peritoneal exudate.

3. Slide of Negri bodies showing their internal structure.

All Negri bodies show inner corpuscles varying in size and number. These

are best shown by the fixation, and staining method used for this slide, i.e., fixation in strong Flemming's solution followed by staining by the iron-haematoxylin process

- 4 Series of slides showing in sections the sandfly *Phlebotomus argentipes* in the act of feeding

The slides exhibit the stream of blood flowing up the proboscis, the arrangement of the mouth parts during the act of feeding and the extravasation of blood at the tip of the proboscis

- 5 Exhibit to show the contrast between concentrated and unconcentrated preparations of protozoal cysts in faeces

The concentration method is that in use in the Parasitology Department of the London School of Hygiene and Tropical Medicine, and is a modification of Faust's method. Concentrations of twenty to forty times are obtained

- 6 A series of exhibits illustrating endemic human fluorosis in India and China, and experimentally produced fluorosis in animals in India and England

Endemic fluorosis was first described by H. E. SHORTT, C. G. PANDIT and T. N. S. RAGHAVACHARI in 1937 in Madras Province where it was found in a severe form in certain cases. The exhibits illustrate this condition in India as it occurs in human cases. Its occurrence in Kweichow Province is illustrated by the exhibits of Dr. O. LYTH while exhibits of human and animal teeth by Dr. M. M. MURRAY and Dr. D. C. WILSON show the dental condition produced in England from the same cause. The remaining exhibits illustrate the condition as produced in experimental animals in India and in England, by the addition to their diet of fluorine in different quantities and by different methods

#### Dr. J. J. C. Buckley (Helminthology Department)

- 1 Incidence and distribution of human helminths in North Eastern Rhodesia

The results of helminthological survey in Northern Rhodesia were summarized in the form of a series of maps illustrating the percentage rates of infection and the distribution of the principal human helminths in the Northern Province.

- 2 Bilharziasis in human bladder

An example of gross lesions of the bladder caused by *S. haematobium* in a native of Northern Rhodesia.

- 3 Experimental infection of *Simulium neavei* with *Onchocerca colvulus*

*Simulium neavei* was experimentally infected with *Onchocerca colvulus* in Nyanza Province Kenya. Stained sections of this vector killed at varying intervals after an infective blood meal, show the developing larvae of *O. colvulus*

- 4 Original preparations of Prof. A. Looss demonstrating the migrations of hookworm larvae in a dog (Exhibit kindly lent by Prof. R. T. Leiper)

Dr G Macdonald, with Mr H S Leeson (Entomology Department).

Demonstration illustrating malaria in the Levant.

The exhibit referred to the Lebanon, Syria and Palestine, which constitute a single geographical unit and consist essentially of a very narrow coastal plain, a coastal range of mountains in some places reaching 9 000 feet, a rift valley—the base of which varies in altitude from 3 000 feet above to 1,300 feet below sea level—an inland range of mountains and to the east of this a vast expanse of desert. Rainfall decreases from the west to the east, and on the whole the temperature is suitable for the transmission of malaria from about May to October. Maps which were shown illustrated these features and marked the most malarious areas.

A series of photographs illustrated normal and abnormal breeding places of the four malaria carriers. *Anopheles sacharovi* typically breeding in swamps and present almost throughout the coastal plain and the rift valley. *Anopheles superpictus* typically breeding in shingle-bedded streams and scattered throughout the two mountain ranges. *Anopheles claviger* typically breeding in the hot weather in subterranean waters and, therefore, confined as an important agent to isolated localities where subterranean storage is common. *Anopheles stephensi* present only in the eastern part of the area and breeding in minute seepages and streams. Adult haunts were also illustrated. All these four mosquitoes infest houses, stables and tents.

The chief method of control was by site selection based on maps prepared after detailed survey of the entire country. The prevention of breeding was in the hands of "Anti malarial Control Units" under the general supervision of a Malaria Field Laboratory. Much reliance was placed on water management, canalization, drainage, flooding, flushing and such like measures, and on larvicides—the principal one used and illustrated in the exhibit was parva green and water by the method of Aziz (1939) which proved the best available for the typical *superpictus* breeding place. Suppressive treatment was only resorted to in a few isolated troops, and a few groups along the Turkish frontier affected by breeding on the other side of it.

The result of control was illustrated by a histogram giving the monthly instances of cases in Ninth Army which amounted to a total of 43.5 per 1,000 per annum.

Dr R F Tredre (Uganda)

*Anopheles gambiae* var. *melas* as a factor in the incidence of malaria in the vicinity of Freetown Estuary in 1942

By charts, diagrams and photographs the following items were demonstrated —

1. The difference between the egg and larva pecten of *Anopheles gambiae* and *A. gambiae* var. *melas* as described by MUIRHEAD THOMSON and RICHARDS respectively

2. The Mangrove Belt and the particular breeding places of *A. gambiae*

var *melas* in Avicennia Mangrove Salt Marsh, the distribution of Avicennia Mangrove along the shores of Freetown Estuary

3 Anopheline vector indices and comparative numbers of *A. gambiae* *A. gambiae* var *melas* and "other" anopheles caught at groups of catching stations on the southern shore of Freetown Estuary between Aberdeen and Waterloo during 1943

4 Dissection results for *A. gambiae* var *melas* (1 000 mosquitoes) were Sporozoite rate 4.2 per cent, oocyst rate, 4.7 per cent., total infection rate, 7.8 per cent

5 A permanent method of control tide exclusion by embankment construction at Aberdeen. Reduction in *A. gambiae* var *melas* population of Aberdeen village shown by catching station results for the years 1941 1942 and 1943

Lt-Col W R M Drew and Major Kendall Dixon

Three methods were shown for making blood lipid estimations after fatty test meals, in cases of sprue

- (a) Opacity of the serum. (b) Chylomicron counts of capillary blood.  
(c) Chemical methods

Dr F Hawking

- (a) Cultures of *Plasmodium relictum* (b) *Leishmania* in tissue culture

Dr William Hughes and Dr R B T Baldwin (Nigeria)

Slides and photographs illustrating nutritional deficiencies (ariboflavinosis and achromotrichia)

- A.1 Photographs demonstrating the connection between ariboflavinosis and kwashiorkor  
2. Sections of liver showing fatty infiltration.  
3. Sections of skin from kwashiorkor showing parakeratosis.  
B.1 Photographs of children suffering from nutritional achromotrichia ? pantothenate deficiency  
2. (i) Hairs showing loss of pigment in acute stage (ii) Hairs with pigmented roots taken during recovery

Major E Geal

Six freak ' malarial slides from the teaching material of the Royal Army Medical College. The slides were chosen to illustrate the difficulty of microscopical diagnosis.

Mr J H Grundy

Drawings of entomological specimens

An exhibition of black and white original pen drawings part of a series

designed to illustrate the life histories of arthropods of medical importance. Most were drawn from living specimens, all to the same scale of 16 diameters.

Those shown were —

- 1 *Anopheles maculipennis atroparvus* VAN THIEL, 1927
- 2 *Aedes aegypti* (LINNAEUS 1762).
- 3 *Pediculus humanus capitis* DEGEER, 1778.
- 4 *Pediculus humanus corporis* DEGEER, 1778
- 5 *Phthirus pubis* (LINNAEUS, 1758)
- 6 *Xenopsylla cheopis* (ROTHSCHILD 1903)
- 7 *Cimex lectularius* LINNAEUS 1758.
- 8 *Sarcoptes scabiei* (LINNAEUS 1758)
- 9 Some wings of Calypterate flies.

Dr B Malamos (shown by Col H E Shortt)

Photographs illustrating pellagra and famine oedema as seen in Greece during the German occupation

Demonstration, by means of a series of twenty three photographs, of the widespread results of famine conditions in Greece during the German and Italian occupation of that country. Three predominant types of malnutrition were represented but these merged, one into another so that the lines of demarcation were not sharp or clear cut. The types were (a) General oedema (b) General emaciation. (c) Great emaciation with ascites.

In children the emaciation was very pronounced as shown by the wrinkling of the skin on the limbs.

The later photographs showed the improvement produced as a result of rehabilitation measures, in spite of the difficulty of obtaining the correct or sufficient food for these measures.

Vitamin treatment, before the supply of adequate and proper food, gave no beneficial results.

As regards pellagra, this appeared at a later stage of the occupation, and only after the onset of milder conditions in spring with an increase of sunshine.

Major Janet Niven

Preparations of rickettial suspensions for the diagnosis of typhus fever.

Rickettial suspensions are prepared according to the method devised by Dr FORREST FULTON of the National Institute for Medical Research, Hampstead. Murine suspensions are made from infected rat lungs inoculated intranasally epidemic (house-borne) from mouse lungs. At harvest, the lungs are almost completely consolidated and contain abundant rickettsiae. After

grinding up the lungs, the rickettsiae are separated from gross particles of tissue by differential centrifugation. The resulting crude emulsion is treated with approximately 6 per cent. kieselguhr (Celite 244 JOHN'S MANVILLE Co) which appears to absorb fine tissue debris, leaving an almost pure suspension of rickettsiae. This is concentrated by centrifugation suspended in a small amount of buffered saline (pH — 7.0) and preserved with merthiolate (1/10 000). The concentrated suspensions are stored at 4° C and diluted for use with physiological saline to match a *Bact coli* suspension of  $500 \times 10^6/\text{ml}$ .

Rickettsial suspensions are ideally suitable for the serological diagnosis of typhus fever but their main use is to differentiate between murine and epidemic (louse-borne) types. Cross-agglutination occurs but the homologous titre is always at least four times as high as the heterologous. The antigen serum mixtures are incubated at 37° C in a water bath for 18 hours and the reading is made under suitable conditions of illumination—a viewing box with strip lighting—immediately after removal from the water bath. Pro-zone formation is very liable to occur with high titre sera and it is important to carry the test to a sufficiently high serum dilution, 1:40 960.

Miss I. M. Tonkin

#### Exoerythrocytic forms of *Plasmodium lophurae* in turkeys

*Plasmodium lophurae* was isolated from the fireback pheasant (Borneo) by COGGESHALL in 1938 (*Amer J Hyg* 27, 615) and was found to give a heavy infection in ducks by WOLFSON in 1940 (*J Parasit* 28, Suppt. 28). The parasite has since been used extensively in America and to a limited extent in this country in the study of chemotherapy of malaria.

In spite of the heavy blood infection obtainable in ducks, all efforts to demonstrate an exoerythrocytic phase of the parasite in this host and in chicks have met with failure.

In 1944 it was heard that Dr R. J. PORTER and Dr LAIRD had found exoerythrocytic forms in turkeys. Accordingly, two turkey chicks, 14 days old, were infected by the intravenous injection of sporozoites of *P. lophurae* from *A. albopictus* mosquitoes: the birds receiving approximately the equivalent of thirty and fifteen infected mosquitoes in 1.0 c.c. and 0.5 c.c. of Ringer/chick serum respectively.

On the 6th day after infection blood smears showed 1 per cent. of the red cells to be parasitized, the birds looked quite healthy but died during the night of the 6th to 7th day. Blood smears taken postmortem showed 7 per cent. of red cells parasitized. Smears of the brain and sections of organs so far examined all show the presence of exoerythrocytic forms. The morphology of these forms closely resembles that of the exoerythrocytic forms of *P. galinaceum*.

These forms have been grown in tissue culture and further details will be published later.

Dr H C Trowell (Uganda)

The kwashiorkor syndrome :

- 1 X-ray pictures of a "deficiency bowel pattern."
2. Photographs showing the response of the dermatosis (present in certain advanced cases) to nicotinic acid
- 3 Liver biopsy sections to show fatty degeneration

The kwashiorkor syndrome (or a "syndrome of malignant malnutrition") is probably the commonest malnutritional disease in Africa, south of the Sahara. Yet it is but little recognized. It has also been reported in the West Indies. Cases have been described in Polish adult refugees.

By courtesy of Dr J SCOTT BROWN X ray films of "deficiency bowel pattern" were shown. This is a fairly constant feature of kwashiorkor. Its significance is but little understood. It must surely reflect an abnormal function of the myenteric plexuses but it may be premature to ascribe this to a deficiency of the vitamin B complex.

By courtesy of Dr JOSEPH GILLMAN and Dr THEODORE GILLMAN, liver biopsy sections showing fatty changes, were shown. Little was known about the pathology of this syndrome for myself I paid little attention to the liver and considered that the fatty change was a terminal change and of little significance. Liver biopsy has demonstrated that fatty liver is an early and constant feature of this syndrome, that it does not respond to nicotinic acid, thiamine, or riboflavin but that desiccated hog's stomach quickly restores the hepatic lesion. In some twenty cases of this syndrome in childhood the clinical response to desiccated hog's stomach has been, in my hands, most gratifying. If their work is confirmed, they have contributed much to our knowledge of the pathology of this syndrome and at the same time its cure. It remains to detect what are the lipotropic substances present in hog's stomach.

Early cases of this syndrome show failure of growth, oedema and pallor of the African skin and hair. Advanced cases show a dermatosis which may reflect a deficiency of some part of the vitamin B<sub>6</sub> complex. Photographs of an adult case, responding to nicotinic acid, were shown.

It is to me a pleasure to demonstrate these specimens to those present and to Dr CECILY WILLIAMS, who originally described kwashiorkor and claimed that it was a new clinical entity. To this view I now subscribe, although at one time our views appeared divergent.

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## ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 13th December, 1945, at 8 p m

THE PRESIDENT

C M WENYON C.M.G. C.B.E. M.B., B.S., B.Sc. F.R.S.  
in the Chair

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## PAPER

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### TEACHING OF TROPICAL MEDICINE

BY

L. EVERARD NAPIER C.I.E. F.R.C.P.

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I have recently returned to England after a continuous absence of nearly five years of which the last two were spent in the United States. In that country a serious attempt is being made both to improve the teaching of tropical medicine to the undergraduate student and to develop postgraduate teaching in this subject. Great Britain is held up as an example in this country, it is claimed, where admittedly we have greater opportunities—and, they seldom miss the opportunity of adding greater responsibilities—for the teaching of tropical medicine, full advantage has been taken of these opportunities. I am afraid that while I was in the United States I was often guilty of perpetuating that misconception. How great a misconception it is, I did not realize until I returned to this country a few months ago. Any lead that we may have held in the field of tropical medicine we are rapidly losing and if a war that was waged largely in tropical regions has failed to stimulate us to teach even the rudiments of the diagnosis and treatment of the commoner tropical diseases to the thousands of students and doctors who are now—or had not Japan collapsed might well have been—serving in the tropics, the outlook for the future of tropical medicine is indeed black.

In this country tropical medicine appears to have been relegated to the place of a specialization about which the general practitioner need know nothing and with which the ordinary undergraduate student should not be burdened. In the *Goodenough Report* for example, the existence of parasites



other than bacteria is apparently not recognized and there is no suggestion that the undergraduate student need be introduced to diseases which are not prevalent in his own country whereas under the heading of postgraduate teaching, clinical tropical medicine is dismissed as a subject that cannot possibly be taught in this country.

In the training of the physician (*sensu lato*) it seems to me very doubtful if tropical medicine should be considered as a specialization at all, in the sense that is radiology ophthalmology or even paediatrics in which special studies are made in limited fields. The science of medicine is concerned with the study of Man, in his reactions to parasitization, to his environment and nutrition, and to degenerative processes. This is true of medicine in the arctic regions, in the temperate zones, and in the tropics. The external influences to which man is subjected may be different in each case, but, as man and the general principles of his reactions remain essentially the same, different approaches do not seem to be justified. By confining our studies to man's reactions to one group of parasites the bacteria, and to a comparatively standardized set of environmental and nutritional conditions, we are limiting the scope of the study and thereby stultifying medical science. What we can learn from man's reactions to conditions that prevail mainly in the tropics will frequently be of the greatest value in our studies of diseases encountered in the temperate zone. Further many new lines of thought have actually been started by investigations in the tropics. Perhaps one of the most striking examples is beriberi and the vitamins. Even today vitamin deficiencies can be studied in their crudest and most easily recognizable forms in tropical populations and as a result serious deficiencies, that are so frequently masked when they occur in the temperate zones, are now more easily recognized and readily corrected.

Any liberalization of the outlook in medical science cannot start and finish in the research laboratory but must be fully appreciated by the teacher and installed into the student, if it is to be real. Further teaching and research should always run along more or less parallel lines. It seems to me therefore to be entirely wrong to segregate so-called tropical medicine in the way that it has been segregated in the past, to introduce the student to the bacteria only amongst the pathogenic parasites, and to teach him virtually nothing of the pathology symptomatology and therapeutics of tropical diseases.

#### UNDERGRADUATE TEACHING.

There are of course practical difficulties to be overcome. The most important is the danger of further overloading the curriculum. However, I feel that much could be done to obviate this by introducing the student to the pathogenic microorganisms and worms and teaching him something of the systematics, morphology and physiology of selected representatives of the parasites of man, in his premedical years and by extending the teaching of bacteriology to include all the microorganisms and worms that commonly

parasitize man (and incidentally by changing the name of this subject from bacteriology to parasitology) The important and essentially clinical diagnostic methods used in tropical medicine should be included in the teaching of clinical pathology This will leave the pathology, symptomatology, and therapeutics of important tropical diseases to be taught in the systematic lectures on medicine (where these are given, or in a special short course of lectures, where they are not) and in the wards and out patient department when the occasion arises Finally, tropical diseases present excellent examples for the teaching of the general principles of preventive medicine

A difficulty will arise in connexion with material for the practical classes in parasitology and for the teaching of clinical pathology and histology In the United States this difficulty was faced very early in the war, the Army through the Army Medical School at Washington, undertook to obtain and distribute suitable material to all the medical schools in the country nominally on a reciprocal basis Actually at first they gave out about ten times as much as they received from the other schools but later some of the schools through their tropical connexions, were able to contribute much material to the common pool, and the organization is now receiving an abundance of material from the thousands of students who have been through the two-months' course of tropical medicine at the Army School and who are now serving in various tropical fields Whether the Army will continue to undertake this task after the war does not seem certain but they have not yet indicated that they will discontinue it The scheme has proved so valuable that it will undoubtedly be continued after the war even if the work has to be handed over to some other organization.

The teaching of tropical pathology in the medical schools is also aided by another Army organization the Army Medical Museum prepared sets of lantern slides of over a hundred subjects mostly histological sections but a few gross pathological specimens and clinical subjects were included. A list of these slides was sent to all the medical schools, and the slides that they asked for were sent to them free They were also given to other persons engaged in teaching tropical medicine I was myself given as many as I asked for several dozen and I now regret that I did not ask for more. Later this museum prepared another set of about 120 slides mostly in colour Of these they prepared only about a dozen sets as they were more expensive to make they did not give them to the schools but lent them for periods of up to three months

One could not reasonably expect the same degree of co-operation from the Army here, as the services have not shouldered the responsibility for undergraduate medical education in the way that they have done in the United States but it should be possible to establish an organization, similar to that at the Army Medical School in Washington at some other institution in this country I am speaking without any authority but I believe that work of this kind would fall within scope of the Wellcome Foundation

The next difficulty is the scarcity of clinical material The importance of clinical teaching cannot be over emphasized but the scarcity of clinical material

must not be used as an excuse for complete inaction. During his two years of clinical work the student expects to see examples of most of the common diseases of the country in which he is working but he will not see examples of many others which he will be expected to diagnose and treat should he encounter them later in his practice. Among the latter are certain infectious diseases, such as typhoid and smallpox, which today are so rare in sanitariously advanced communities that the average student never sees them, yet nobody would seriously suggest that they be omitted from the curriculum. Surely we should adopt the same attitude towards tropical diseases. In those schools in which there are systematic lectures, the important tropical diseases could be covered very adequately with the aid of a good set of lantern slides, preferably in colour, or better still with cinema films, but a very great deal can also be done in the wards if the teacher himself is sufficiently conscious of tropical diseases. Too frequently he is not. I have seen a first year clinician and teacher discuss at great length a case of undiagnosed splenomegaly without mentioning malaria, kala-azar or schistosomiasis, and in another instance in a case of chronic ulcerative colitis amoebiasis was peremptorily dismissed on a single negative stool examination, though later the condition cleared up entirely after a single course of emetine. Frequently the fault lies in the teacher's complete ignorance of even the existence of many tropical diseases, an ignorance of which too often he is apparently more proud than ashamed.

It will be difficult to adjust this defect suddenly, however an excellent opportunity will arise during the next few years when not only will there be an increasing number of cases of tropical disease among the personnel of the forces and among civilians whom the war has taken into the tropics, but there will be many physicians returning from service in the East who will have acquired an interest in tropical problems and a certain number will be very ready to maintain their interest after their return to this country. This will undoubtedly lead to a temporary stimulation of interest in tropical diseases throughout the country but the problem will be to keep up this interest. It is certainly true that air travel has made the world smaller, that persons returning to or visiting this country by air from the tropics will arrive well within the incubation period of almost all tropical diseases, and that therefore in many instances persons with tropical diseases will pass the most vigilant airport health authorities and when they become ill will seek the advice of their own practitioner on whom will fall the responsibility of making a diagnosis. But on the whole such instances will be rare in the experience of any one doctor, and it seems doubtful if they will provide a sufficient stimulus to maintain the interest of the medical profession in tropical diseases unless this interest is fostered in some other way, naturally or artificially.

Perhaps the easiest of the natural ways of fostering this interest will be by involving the home research worker in tropical problems and encouraging a closer collaboration between research workers in Great Britain and those working in the tropical fields, in the Colonies, in India and Burma, in the Dominions.

and in other tropical countries possibly with arrangements for short term exchanges of personnel. There are many tropical problems of which the basic experimental work (e.g. synthetic drug chemistry and pharmacology) could be carried out satisfactorily in this country even without clinical material in other instances a well-organized 'tropical diseases centre' in London or Liverpool would provide all the clinical material necessary for preliminary investigations and in yet other instances the work may have to be done entirely in the tropics. Wherever the work is done the practical applications will have to be in the field, and although there are obvious advantages in having as much of the work as possible done in Great Britain if the home worker is not familiar with the tropical conditions in which it is to be applied, it will be essential that at all stages a very close liaison be maintained between the home and the field workers.

I am not now considering medical research as such, but only in its rôle as a sustainer of the interest in tropical disease of the physician and teacher in Great Britain and therefore I feel that the greatest advantage will be achieved if home research workers on tropical problems are mostly associated with teaching institutions. In the past the emphasis in tropical research has been on the aetiology and prevention. In such investigations the major rôles have been played by the parasitologist and entomologist whose counterparts in this country have seldom been closely associated with undergraduate medical schools. There are of course many aetiological problems still to be solved, but the centre of interest is now tending to swing towards chemistry pharmacology biochemistry and clinical pathology other than parasitology and there is certainly room for much more research in physiology and histopathology all familiar subjects to the teacher and student in medical schools. This will all help to narrow the gap between so-called tropical medicine and let us call it temperate medicine, and to make the home physician realize that tropical diseases are after all not so very exotic in their pathology and symptomatology even if their modes of transmission are somewhat complex and unfamiliar that one can take an intelligent interest in them with little knowledge of that very foreign subject, entomology and that there is nothing essentially improper or even funny in mentioning the name of a tropical disease to the undergraduate student, when the occasion arises which it frequently does, in almost any discussion on differential diagnosis for example.

Sir WILSON JAMESON has suggested that, if there is to be a state medical service, it might be possible to arrange for the personnel to do short terms of service in the Colonies. This would certainly broaden the outlook of a large number of medical men, as well as being very beneficial to colonial medical practice, but it would be even better if the suggestion could be extended to include prospective holders of teaching appointments in medical schools.

As an artificial way of maintaining interest in tropical diseases I would suggest that examining bodies especially those concerned with the higher

qualifications which teachers and consultants are expected to possess such as the college memberships, should demand a sound knowledge of at least the commoner tropical diseases.

### POSTGRADUATE TEACHING

In this connection we in Great Britain still hold all the aces, but it is our apparent disinclination to take advantage of the position and to play our cards properly that is so disconcerting. It would indeed be a tragedy with considerable political and financial as well as medical repercussions if we were to allow America or any other country to take the lead in the postgraduate teaching of tropical medicine and to attract to their country doctors and even patients from the Dominions, the Colonies, India and foreign countries who would otherwise have come to London or Liverpool.

In his Memorandum drawn up for the Council of this Society Brigadier HAMILTON FAIRLEY told what was being done in other parts of the world, including America. I think that I have a little more intimate and certainly more recent knowledge of what is being done there. Although the plans for developing tropical schools in New York and San Francisco are considerable, they have not yet been developed to any very great extent. I have little doubt that these plans will eventually mature but in the meanwhile we have the very great advantage of already having two experienced staffed and equipped schools in England, as well as the smaller schools in Scotland. However a medical school without a close hospital association would be nothing better than a cramming institution and would not be allowed to exist in Great Britain (or America) today. Similarly no school of tropical medicine can hope to be a living and progressive institution without clinical material for research work and for keeping alive the interest of those engaged in teaching, perhaps even more than for the demonstration of cases to students and as a source of fresh material for laboratory classes although the latter two are themselves sufficient reasons for the demand for a tropical diseases hospital.

I understand that in Liverpool a satisfactory though possibly temporary arrangement for clinical material has been worked out. In London there is an abundance of clinical material which will certainly show a marked increase during the succeeding years, but at present there is no hospital where it can be collected, and I am entirely in agreement with our President and Brigadier HAMILTON FAIRLEY in their suggestions that our most crying need today is a tropical medical centre. To such an institution, all sick persons returning from the tropics could go for diagnosis and treatment, whether they were suffering from an obviously tropical disease or from some other condition. Here should be available not only the services of experts in various branches of tropical medicine but also those of general physicians, surgeons and specialists in other branches of medical science. For this reason the tropical medical centre should, in my opinion, be closely associated with a medical school. This

contact would be of the greatest benefit to the staffs of both institutions (and thus indirectly as well as directly to their students) since if the home physician is liable to forget the existence of tropical infections the tropical physician usually tends to investigate his cases on rather set lines and sometimes fails to take the fullest advantage of modern methods. Whether it would be possible to persuade the Army the Navy and the Air Force to co-operate in such a scheme, though obviously it is desirable that they should, seems uncertain but even without them it will be possible to maintain a very active centre with a large variety of tropical infections always available. I am here considering the importance of such a centre from a limited point of view. It is also an urgent need from a humanitarian point of view as today the plight of the sick empire-builder returning to this country is really a serious one.

I have said before that there is in London an abundance of clinical material. This will perhaps be an appropriate moment to raise a point regarding a common misconception about tropical diseases namely that in medical practice in any tropical country one will immediately encounter all, or at least a very large variety of the diseases that are usually labelled tropical. Actually there are few if any places in the world where one could see a better selection of tropical diseases than here in London. It seems to me that this misconception is reflected in a sentence in the *Goodenough Report*

This report includes the following paragraph —

While a man may acquire a considerable amount of theoretical knowledge and become reasonably proficient in certain laboratory processes it is abundantly clear that he cannot be given an adequate clinical training in tropical medicine in this country. Therefore we suggest that, having obtained his theoretical and laboratory instruction here, a student wishing to specialize in tropical medicine and hygiene should be required to hold approved hospital appointments in tropical countries as part of the prescribed postgraduate training and experience in this branch of medicine.

What do the framers of this report mean by an adequate clinical training in tropical medicine? Adequate for what? In the average hospital in the tropics a student might see an abundance of cases of one or two tropical diseases and isolated examples of a few others, and, in the case of the former being able to follow them through all their stages he might become thoroughly experienced in the diagnosis and treatment of those one or two diseases as they occur in that locality but when he goes to another tropical country he may well find his concentrated experience quite useless or even misleading and it will certainly not justify his posing as a specialist in tropical medicine.

The object of a course of tropical medicine should be not to make the student an expert in all tropical diseases but to equip him with additional knowledge, which time did not allow him to acquire during his ordinary medical course in order that he may take full advantage of the opportunities to become an expert that will be provided in his subsequent practical experience in

whatever tropical countries he visits. Clinical instruction should play an important part in, but should not dominate, this course. The postgraduate student should already possess a sound knowledge of case taking and physical diagnosis and if he does not, a course of tropical medicine is not the place for him to acquire it. The approach to the patient should be very much the same in the tropics as in a temperate climate, and of course, as always, the approach will have to be modified according to the circumstances in which the patient is seen. Actually to the student in a clinical lecture the value of the exhibition of one hospital case at one stage of the disease, though considerable, is mainly psychological, whether the hospital is in London or in Sierra Leone, because the picture that the patient presents is often so far removed from that of the typical case encountered in its natural environment that it may be actually misleading.

However if any university college or school wished to institute a higher diploma in tropical medicine which would give the possessor a specialist or consultant's status (I seriously question the value of such a project), then I would suggest that they should demand in addition to the above course, several years' not months' experience under approved supervision, and certainly not all in hospitals, in not one but two or more tropical countries, and a period of independent experience in yet another before the candidate is allowed to take his final examination. The main part of this examination should be the presentation of a thesis on an approved subject which must include some independent and original research work.

I am not proposing to discuss here the question of the staff requirements for a school of tropical medicine or the syllabus for an adequate postgraduate course of tropical medicine but, as at last year's Annual Meeting of the American Society of Tropical Medicine I read a paper on this subject, I have brought two slides that I used on that occasion and I will show them.

TABLE I. SUGGESTED SYLLABUS.

1. Climatology and the Geography of the Tropics.
2. Ethnological Studies.
3. Economics.
4. Medical Organisation in Tropical Countries.
5. Public Health in the Tropics —
  - (a) Vital statistics — mortality and morbidity data.
  - (b) Simple statistical methods.
  - (c) Education and propaganda.
  - (d) Housing, hospital construction, sanitation and water supplies, and material engineering.
  - (e) Measures to mitigate the effects of heat, and the maintenance of health in the tropics.
  - (f) Epidemiology.
  - (g) Special measures of disease control.
6. Physiology of Hot Climates.
7. Nutrition and the Common Deficiencies in the Tropics.
8. Zoology.
9. Entomology.

- 10 Parasitology including the study of helminths, protozoa, rickettsiae, spirochaetes, bacteria and other fungi and viruses
- 11 Herpetology
- 12 Pathology
- 13 Haematology clinical laboratory tests, and biochemistry
- 14 Pharmacology and Therapeutics.
- 15 Tropical Medicine correlating lectures on individual diseases.
- 16 Clinical Teaching
- 17 Tropical Skin Diseases
- 18 Tropical Eye Diseases
- 19 Surgery in the Tropics.
- 20 Obstetrics in the Tropics
- 21 Tuberculosis in the Tropics
- 22 Venereal Diseases in the Tropics.
- 23 Psychiatry in the Tropics.
- 24 Nursing in the Tropics.
- 25 Medical Administration in the Tropics.

TABLE II SUGGESTED STAFF

The full time staff should include —

- 1 Professor of Tropical Medicine and one or two assistants.
- 2 Professor of Public Health, and one or two assistants.
- 3 Professor of Parasitology and two or three assistants.
- 4 Medical Entomologist, and an assistant.
- 5 Epidemiologist and Medical Statistician.
- 6 Biochemist.

One medical member of the whole-time staff should have had experience with an industrial concern in the tropics, another should have been a Government servant, and a third should be a woman with tropical experience, the last might well be additional to the above.

(Some of the assistants might also be employed as teachers in the associated under graduate school)

The following subjects should be taught by special assistants in the various departments in the associated medical school —

- |                     |                          |
|---------------------|--------------------------|
| 1 Pathology         | 7 Eye diseases.          |
| 2 Pharmacology      | 8 Obstetrics.            |
| 3 Surgery           | 9 Psychiatry             |
| 4 Tuberculosis.     | 10 Physiology            |
| 5 Skin diseases.    | 11 Nutritional diseases. |
| 6 Venereal diseases |                          |

The remaining subjects should be taught by other members of the staff of the medical school, or university

This scheme was drawn up for a School of Tropical Medicine in the United States and especially for a school associated with a university medical school.

I would just like to add that, although I feel very strongly that all the students who take the course should attend some lectures on all these subjects the time has perhaps come when special provision should be made for those who are proposing to specialize in tropical hygiene such men should be required to attend longer courses in some of the subjects and shorter courses in others, and should also undergo probationary periods with experienced field workers in the country in which they are going to work, or if they are to be given a higher diploma in tropical hygiene, in two or more countries, as was suggested for the higher diploma in tropical medicine.



I have made my remarks as concise as possible in order to allow the maximum time for a discussion on this subject which I feel is a really important one not only for those like ourselves who are especially interested in tropical medicine but for the profession as a whole. I naturally cannot expect that all of you will agree with all that I have said, but I feel confident that some of you will agree with some of what I have said, and I hope that many of you will agree with much that I have said. If my hope is fulfilled I would then like to ask the question "Where do we go from here?" I would prefer that you should all disagree with me entirely than that you should agree in principle and then let the subject drop. If you are not prepared to do anything about this matter I am afraid that nobody else will. This Society has in the past primarily devoted itself to scientific matters and has seldom attempted to influence the policy of the profession. I am very reluctant to suggest that there should be any departure from previous practice, but nevertheless I do feel very strongly that at a time when so many changes are taking place in the profession someone should sponsor the opinions of those of us who are interested in tropical medicine. Surely this could not be done more appropriately than by this Society.

There are two points that I have tried to emphasize particularly this evening namely (i) the necessity for making the undergraduate student conscious of the existence of diseases other than those that occur commonly in his own country and (ii) the urgent need for a hospital in London that will act as a clinical centre for teaching and research in tropical medicine. To this latter point I have devoted less time as the case has already been presented so ably by Brigadier HAMILTON FAIRLEY in his memorandum of 16th January and so forcibly reiterated by our PRESIDENT in his recent inaugural address, but I have felt compelled to raise it again since such action as was taken has not been particularly fruitful. It is a matter of very great regret that Brigadier HAMILTON FAIRLEY is not here tonight however I feel confident that he would be the first to deplore our using his absence as an excuse for doing us any action that might be taken.

I have two definite proposals to make (i) that this Society should prepare a memorandum on the teaching of "tropical medicine" to the undergraduate student and circulate it to universities, medical schools, examining bodies, and others likely to be interested in Great Britain and Ireland and possibly in the Dominions, Colonies and India, and that it should endeavour to stir up as much interest as possible in this memorandum and in the subject generally through the general medical press and by other means and (ii) that this Society should endeavour to obtain direct representation on all committees concerned with the policy of the teaching of "tropical medicine" both undergraduate and postgraduate, or with research in tropical diseases, which of course will include committees concerned with any tropical diseases hospital.

## DISCUSSION

Sir Philip Manson-Bahr There are some points in Dr NAPIER'S paper with which I would agree but I should like to see that much abused poor creature—the medical student—alleviated with regard to his curriculum and not have fresh burdens put upon him. Those who have recently attended the Comitia of the Royal College of Physicians will know what these burdens are—psychology social medicine industrial medicine in addition to others. Are we now going to impose on him the whole gamut of tropical medicine?

With one suggestion which has been made I should like to express my emphatic agreement, namely that the pre medical studies should be rendered more humane, more associated with the diseases of man. Why should not the medical student study the *Entamoeba histolytica* instead of the pond amoeba? Why should he not study an intestinal worm such as *Ascaris* instead of the lowly earthworm? Why should he not consider the crayfish as a vector of important human disease? That would create ideas in his mind which would remain for the rest of his life. It is a mistake to assume that undergraduates in medical schools do not get some modicum of tropical medicine in their studies. They most certainly do.

I myself have been lecturing at the London Hospital to medical students for over 25 years. I know full well that in recent decades both dysentery and malaria have been set as questions in the pass examination for the M.R.C.S. and L.R.C.P. I owe my existence here tonight to the fact that at the beginning of the last war they set me a question on dysentery which I happened to know about otherwise I should not have got the M.R.C.P. mainly because my examiners were themselves ignorant of the subject.

I have been engaged in clinical teaching for a great many years and have risen by all stages in tropical medicine from that of a demonstrator to that of a clinical physician. I beg you not to overload the tropical curriculum that the student becomes so crammed that he forgets those very essentials which he came to the schools of tropical medicine to learn.

On looking back on my career the best days for tropical medicine in London were those when MANSON was at the helm. He and those associated with him turned it into a human affair. They saw that the students knew how to make blood films and recognize a malaria parasite when they saw one and they went on hammering at parasites and intestinal protozoa until the students knew what the *Entamoeba histolytica* looked like and did not forget it.

It is a most important thing when a man knows his microscopic work, but if we make the course too elaborate he tends to forget what he was first taught. I do not believe that the students who have been turned out in recent years are as reliable as formerly in the science of tropical medicine. They have not got the same microscopic background.

On the subject of clinical tropical medicine I should say this. I have always tried to teach it from the differential diagnosis point of view. I have

never looked upon myself as a tropical specialist, or as one who can only look at disease from the tropical aspect. The ideal teacher should be a well-grounded physician—he must know the ordinary diseases and keep himself familiar with the trend of ordinary medicine. If he is introduced to a patient with an enlarged spleen he must be able not only to enumerate the causes of its enlargement, but be able to impress upon his students all the vicissitudes that may arise in differential diagnosis. He must know that kidney disease and liver disease simulate malaria, that people with infected antra or sinuses may develop upon which simulate malaria very closely. He has to sift the wheat from the chaff.

That is the right way I consider to teach clinical tropical medicine in London. We should have, for example, a case of malaria on the one hand and a case of kala azar on the other. We should have a map showing the distribution of these two diseases in order to emphasize the necessity of knowing that each particular disease has a peculiar geographical distribution. Then the student has in front of him a series of slides and reviews a demonstration of the other blood conditions which may give rise to enlarged spleens.

We here in London laboured for 25 years at creating a really efficient Hospital for Tropical Diseases. It got to a certain stage and then it crashed through no fault of my own or that of anybody else in this country. We have to start all over again. But we want a different spirit abroad. There is not one big London hospital where I have not been asked to go to demonstrate a case of tropical disease—liver abscess, kala-azar, sprue, or amoebic dysentery—the students have all crowded round, and everybody has been most interested. Then they have asked me to write out the treatment of the case, and I have said, "I suppose you would not mind lending me this case for one afternoon to teach on?" Their reply has always been, "Oh, no we don't get such a case very often and it is very interesting for our students." I have gone on to ask whether they could let me have it and have been told—only if I paid for the ambulance and all expenses.

SIR ANDREW BALFOUR once said to me, "There is going to be no difficulty about it. All the London hospitals which have tropical diseases will send them to you. What has been the result?"

In 25 years I have only had two cases from outside sent to me. One was described as an interesting case of malaria from University College Hospital, but when the ambulance drew up there rolled out the most obscene, foul-mouthed old sea captain you can imagine with delirium tremens! On the other hand, there do pass through London some most interesting cases. I once saw in Stratford a case of kala azar from China with enlarged glands in the neck. It was impossible to do any more about it, although that man was a scoundrel and I had a perfect right to look after him in our hospital. How are we going to get other people to hand over their interesting cases in the tropical field and get them concentrated into one centre?

There are a great many other things that I should like to say but I do

hope and pray that tropical medicine will remain simplified rather than over-elaborated. There are quite a number of other questions which look very well on paper but do not work out so well in practice and should be left alone.

Prof R M Gordon Dr NAPIER's interesting and stimulating address will no doubt lead to argument, but argument is the sign of a healthy society and although we may differ on many minor points I venture to predict that most of us here tonight are in substantial agreement with the principles Dr NAPIER has expressed. For my own part I disagree with him on two important points. The first is the suggestion to alter substantially the existing D T M & H. courses. the second is that a single great tropical medicine centre be formed in London where *all* sick persons returning from the tropics should go for diagnosis and treatment. But I shall return to those two points later.

Dr NAPIER has suggested that this Society should try to influence the policy of the profession as regards the teaching of tropical medicine. I agree and would propose that it recommend to the General Medical Council that when the medical curriculum is reorganized provision should be made for the teaching of elementary tropical medicine, particularly malaria and the dysenteries in the final years and that in the first years the zoology course might be altered to include the human parasites. While agreeing with Dr NAPIER's suggestion I am glad that he has drawn attention to the danger of trying to add anything approaching a comprehensive course of tropical medicine to the existing undergraduate curriculum. Most teachers find that with increasing experience of teaching their subject, the tendency is to present less and less material to the student and spend more and more time in instilling it. A small amount of fundamental knowledge, well absorbed, is of some real and permanent value while an ambitious and extensive programme rapidly passed over leaves with the passage of time, but little residue of deposited information.

Some of my colleagues in Liverpool who like myself are concerned with the teaching of parasitology to veterinary students, have had experience of the unfortunate results of attempting to crowd more information into an already overburdened course. For example in the veterinary curriculum for the 4th year which deals with parasitology an attempt is made to cover the main parasites of domestic stock throughout the world, with the result that the student graduate lacks a sound knowledge of the much more limited range of species which he is certain to meet with in his everyday practice in this country. Year after year we have observed this failure, and we believe that the sooner tropical veterinary parasitology as at present taught becomes part of postgraduate study the better.

Let us now consider postgraduate teaching in tropical medicine. Before doing so it is necessary to make clear that whenever reference is made to the teaching of tropical medicine it includes tropical hygiene, which, in my view cannot be separated from it.

What subjects should be included in this postgraduate training in medicine

and hygiene and what period of time is required for their assimilation? Dr NAPIER has shown us his suggested syllabus for "an adequate course of tropical medicine," without telling us its duration, but when he showed this programme in America he stated that "a course of about 8 or 9 months, with about 100 hours of instruction, would be appropriate." I believe that his estimate of the length of time required for such a course errs on the short side, and that at least a year or 18 months would be necessary and I disagree with his suggestion that such a course is appropriate for the newly qualified doctor about to practice in the tropics. To my mind the instruction of the graduate student in the country prior to his departure overseas to take up an appointment in the tropical field, should be nothing more than a rectification, in a period of some 4 or 5 months of the essential deficiencies in his general medical curriculum prior to qualification. In my view the courses for the D.T.M. & H. will fit this purpose and I would be reluctant to increase their duration or materially alter their substance. These courses do not result in the production of a specialist, and here we are at complete variance with the *Goodenough Report* but they *should* produce a man qualified to be a practitioner of medicine in the tropics. Armed with this fundamental basis of knowledge, he can then be attention to specialism of various kinds, and the type of specialism he *would* to pursue will dictate where he should undertake further training.

I come now to the second point on which I differ fundamentally from Dr NAPIER. In considering the memorandum by Brigadier HAMILTON FAIRLEY the recent presidential address of Dr WENTON and the utterances of Dr NAPIER and Sir PHILIP MANSON BAKER, one is struck by their apparently unanimous verdict that the position of tropical medicine in London is a parlous one, albeit, I trust, only temporarily so. But each of these speakers, well qualified by local knowledge to express an opinion, goes a great deal further than this, and either expressly states or implies that therefore the position of tropical medicine throughout these islands is in an equally unhealthy state and that the whole picture and prospect of tropical medicine is a dull and gloomy one. One or two of these authorities stated that they "believed there were some clinical activities in Liverpool." I would assure them that their suppositions are correct, and that the oldest school of tropical medicine in the country has flourished and is thriving now and that we in Liverpool have every confidence in her ability vigorously to continue to flourish when the present abnormally large demands on her services have been met. That her energies have not been exhausted in coping with an extensive teaching programme and in dealing with some 5 000 in-patients and with 20 000 out-patient attendances in the last 3 or 4 years, is borne out by the volume of original work produced by the staff of the school. Numerous scientific papers have been produced by the staff many of them leading to important advances.

While I very sincerely hope that the necessary clinical facilities so badly needed in London will shortly be forthcoming I cannot believe that the only salvation for tropical medicine in these islands and the Empire is wholly

dependent on their materialization. It appears to me fundamentally unsound to focus all the resources financial and otherwise, in a single institution. Multiplication of adequate facilities for the investigation and treatment of tropical disease can benefit both patients and workers in the various departments of the subject of tropical medicine. To my mind the expenditure of considerable sums of money in furthering the cause of tropical medicine in this country should be directed towards the support and sustenance of the various existing organizations and not solely to the furtherance of one single project.

May I refer to two other aspects of the subject not fully dealt with by Dr NAPIER? I was impressed by his remarks concerning the availability of teaching material in the United States and how it was supplied to the training centres through the Army organization. I do not think it would have proved possible for the British Army to afford a similar service but I do know that throughout the war the Liverpool School of Tropical Medicine supplied parasitology material for teaching purposes to amongst others two prominent London institutions. In this country there is a great dearth of material for teaching purposes particularly of living material for parasitology and entomology courses. In the early days of tropical medicine there was little demand for such material and students were taught parasitology almost entirely from dried and withered specimens. Nowadays there is a growing tendency to supply the student with live specimens of the pathogenic protozoa and of their vectors thus turning a museum into a zoo to the great advantage of all concerned. The maintenance of these strains involves much labour and care from a specially trained staff and the loss of a strain is disastrous unless it is being maintained in more than one institution. Might I venture to suggest that some organization such as the Wellcome Foundation should undertake the benevolent task of maintaining these creatures and supplying them when needed to the institutions responsible for the teaching of parasitology to medical and veterinary students?

There is another point to which Dr NAPIER has made no reference but which I regard as of considerable importance. At the present time the Colonial Office insists that any medical officer continuing in their service should possess the D.T.M. & H. while an increasing number of firms and certain of the missionary societies have similar rules. Nevertheless at the present time there are a considerable number of newly qualified medical men proceeding to practise in the tropics without any such qualification. Such practice is against the interest of the country in which they are employed. Surely this Society might bring its influence to bear on this as well as on the other problems mentioned by Dr NAPIER.

Dr George Macdonald. It has been most stimulating to hear the constructive criticism of such an authority as Dr NAPIER, particularly with his recent experience in the United States as a background, and I belong to the

group which agrees with much of what he has said, especially his recommendations for the broadening of the basis of undergraduate instruction after Sir PHILIP MANSON BARR's testimony to its possibility and actual practice in many medical schools.

Dr NAPIER told us that it would be tragedy if interest in the teaching of tropical medicine should be lost. Outside the special group of those intimately concerned in it, interest has been lost, lack of it being shown by the failure of the writers of the *Goodenough Report* even to consider it seriously and by the way in which Brig HAMILTON FAIRLEY's memorandum has been allowed to wither and, so far as we know die. This Society has rightly stimulated discussion and as a result three proposals have been made for the replacement of the facilities lost in London as a direct result of the war. Brig HAMILTON FAIRLEY recommended an "Imperial" hospital in association with the London School of Hygiene and Tropical Medicine and the Wellcome Research Institution. Dr WENTON has recommended the foundation of a totally new School, or at any rate the divorce of the present School of Tropical Medicine from the School of Hygiene and its re-establishment around a new hospital, and Dr NAPIER has today advocated the formation of a Tropical Centre similar to that proposed by HAMILTON FAIRLEY but associated with a general hospital.

Any of these proposals would, if carried into effect, involve some considerable modification of the present London School. Though I do not speak as a nominated representative of the School, I might make it clear that the extent of the bomb damage it received is relatively small, and has not interfered with the activities of the tropical section—the pressure on space is no more than normal in any growing institution. I myself have not been able to notice any tendency for the Hygiene departments to squeeze out those interested in tropical diseases and also in my opinion the association between the Tropical and Hygiene departments is one to their mutual advantage. Divorce, as suggested by Dr WENTON is not justified, but extension in the form of additional facilities for clinical and chemotherapeutic research should be considered in association with a new hospital.

An "Imperial" project suggests some exclusive function relegating to a secondary role other centres both in Great Britain and abroad. Certainly Liverpool would have grounds on which to oppose such a proposal, and I do not think it would be regarded entirely favourably in the House of Commons unless they were given an active share in its initiation and subsequent running. Therefore, I suggest that we must have some centre of clinical research in London to replace that lost in the war but on a better and more fully equipped scale than that it should work in co-operation with the existing School of Tropical Medicine as now constituted, and that it should win its "Imperial" pre-eminence by its activities and not by any exclusive title or charter.

Dr NAPIER has given an outline of the postgraduate teaching with which

we must all agree in principle. To elaborate that principle with some details we should emphasize that the course is intended to round off a general medical education rather than to create a specialist, and that the clinical and parasitological subjects should be closely combined with the teaching of preventive medicine rather than separated as has been the custom until recently in Great Britain. The suggested period of instruction given by Dr NAPIER in his American paper was about 1 000 hours spread over 8 to 9 months but I suggest that this is excessive for the purpose for which the course is intended, and that it should be restricted to 4 or 5 months and 500 to 600 hours teaching. I would also like to see some form of machinery instituted to ensure uniformity of standards not only in teaching but also in the examinations of the various bodies.

Dr NAPIER considers that some special provision should be made for those who are proposing to specialize in Tropical Hygiene. I consider very strongly that such people should have the Diploma in Public Health which provides the necessary broad basis of understanding of the principles of preventive medicine and that it can be adequately rounded off for tropical purposes by the D.T.M. & H. provided that it is followed by a period of work under an experienced colleague overseas before the holder takes up independent work. Unlike him, I do think that there is some point in the institution of a qualification for the specialist in Tropical Medicine based on some such standards as those that Dr NAPIER suggests and including an examination ensuring a high standard in general clinical medicine of the type we should call cosmopolitan.

Dr NAPIER asks: Where do we go from here? I hope the Society implements his two recommendations and institutes machinery which will ensure continuity of interest in medical policy which should be framed to take account of the views of workers in Liverpool, Edinburgh and elsewhere, as well as those in London. It might take the form of a Policy Committee: the first duties of which would be to examine and erase the divergencies of opinion about the correct line of development in London and elsewhere to guarantee this country's continued pre-eminence in tropical research, and also, of course, to follow up Brig HAMILTON FAIRLEY'S memorandum, discover its fate after the time when the Colonial Secretary offered the necessary money to implement it, dissent it, and see that it receives the attention it deserves. Secondly the Committee should agree on, and press for acceptance of standards of teaching and examination for both general practitioners and specialists in medicine and hygiene. By such an action the Society could make another very real contribution to the progress of Tropical Medicine and Hygiene.

Lieut.-Colonel E. H. Vere Hodge. Dr NAPIER, in his address, has outlined a forward policy so ably and completely that there is little room left for anything but enthusiastic support. There are, however, points which arise in connection with the present necessitous situation and the urgent demand for men with some, though possibly short, training. Admittedly a central imperial school



of tropical medicine and research must be the ultimate object but however quickly such a scheme matures, interim and immediate measures are required. Large numbers of men are returning to this country suffering from acute disease or latent infection calling for investigation and treatment. Even in this epoch of regimentation it is not feasible or desirable to direct them all to one institution. These men have been exiled from home for some years, many of them have been prisoners they do not want to be collected, they want to be treated near their own homes. Any form of treatment, however scientific will be stultified if it is combined with an impression of coercion and sequestration. Consequently the necessity continues for tropical disease units, with experienced staff attached to general hospitals throughout the country. Such units will do much to solve one of the main problems, the stimulation of undergraduate teaching. All schools for the teaching of Tropical Medicine in this country must labour under some handicap. Clinical material is limited to certain types of disease only and acute primary disease will be absent from the wards. Further treatment is carried out in climatic conditions other than those in which the disease is generated and the patient is more or less assured against re-infection. Consequently primary teaching in this country will demand some implement by the establishment of centres in selected tropical areas. It is to be accepted that such centres could show a smaller variety of disease than could be seen at the main centre in this country but practical experience, opportunities for studying the language customs and local conditions, would compensate for any possible lack of variety. Teachers called upon to lecture on acute diseases find their impressions grow dim if anchored to schools in this country and would be glad to renew atmosphere.

At Edinburgh University during the War the Diploma course was dropped and certain other courses, designed to meet existing needs, were instituted. Firstly in common with other Schools, classes of 14 days intensive instruction were held for officers of the R.A.M.C. Subsequent enquiry from officers who had attended various schools showed that, on taking up practice in the tropics, they found themselves to have advantage over those who had received no such training. Secondly a modified course of 3 months was held for qualified men about to take posts under the Colonial Office. This was arranged in such a way as to be equivalent to and qualify for Part I of the Diploma. Finally at the request of the War Office, a series of lectures was given to undergraduates of the Senior Training Corps. This course was not an outstanding success as students clearly regarded that subject as outside the curriculum and not likely to promote success in the final examination. In this view incidentally they were mistaken, as an extern examiner set a question on the treatment of malaria with catastrophic results. Experience from these courses has led me to the conclusion that teaching activities should not be solely conducted with a view to the Diploma. I suggest that there should be courses of three types. Firstly as stressed by Dr NAPIER, undergraduates

training. Instruction must be included in the curriculum in those diseases now being imported which may be active subject to relapse or lie latent. With the return of many medical men of considerable tropical experience, it is hoped that the attitude in general hospitals to tropical disease will change the staff will include men competent to deal with such diseases to instruct students from first hand knowledge and as already mentioned, special units will be constituted. The regular appearance of questions on the commoner tropical diseases in the final examinations will further stimulate the interest of students.

Secondly a 3 months course for the benefit of those proceeding abroad for the first time. In the light of Dr NAPIER's paper this may seem a retrograde step but present conditions demand it. The necessity for men overseas will prevent any longer training at the moment. It is a short course and with the imposing number of sections involved, such as parasitology, entomology, epidemic and vector control will require careful balance to afford the best practical initiation into tropical practice. In the next course at Edinburgh, I propose that the class shall spend two mornings a week at the Tropical Diseases Unit, which is jointly sponsored by the Department of Health for Scotland and Edinburgh Corporation, where ample clinical material is available and in the laboratory attached so that they may interest themselves in first hand clinical observation and diagnosis. Clinical demonstrations in hospital for tuberculosis and venereal disease have also been arranged.

Systematic lectures must play an important part in instruction especially with reference to the medical emergencies as for instance heatstroke and blackwater fever, the acute fevers, the co-ordination of experience gained in temperate climates with tropical practice and the normal progress of disease under treatment. Finally some attention must be paid to the feeding and care of infants in the tropics.

Thirdly the course for the diploma. I understand that it is possible at all Schools for a candidate to obtain a diploma before ever leaving this country. This should not be so, the diploma clearly implies that the holder is specially qualified for tropical work. Part 1 may be regarded as the preliminary training but a candidate should not be admitted to Part 2 until he has done at least one tour of service abroad and can produce evidence of practical experience. I would like to see the examination for Part 2 include a short thesis or essay on a subject chosen by the candidate showing evidence not necessarily of original work, but of personal observation. Some optional deviation from the standard course is desirable to enable a candidate to make special studies in a branch which he may have selected by this time.

Finally I would refer to the *Army Medical Department Bulletins* and suggest that a similar issue, by a central body for the benefit of medical officers in remote areas would be of great value. Perhaps the Committee of the *Tropical Diseases Bulletin* could be induced to consider the matter.

Lieut-Colonel W R M Drew These two problems, postgraduate and undergraduate teaching have to be considered, and perhaps what I am going to say is in the nature of looking back a little. There has been undergraduate teaching in tropical medicine in the medical schools of London for many years past. In peace-time every school, I think without exception, had a lecturer in tropical medicine, but when the war began this teaching ceased. Some of those present may not know that in 1942 the Director General of the Army Medical Services wrote to the Presidents of the Royal College of Physicians and the Royal College of Surgeons requesting that undergraduate teaching in tropical medicine and pathology very important in war time, should be given a better place in the curriculum.

As a result of that I was asked to give a series of short courses at the Medical Society of London's rooms in Chandos Street. In all I gave eight such courses during the war with a voluntary attendance at each of about 120 senior medical students. Those of you who are familiar with the final examinations of the London, Cambridge and Oxford Universities and of the Conjoint Board, will probably have noticed that there was included at least one tropical question in every examination. No doubt this acted as a stimulus. Certainly I was greatly impressed by the interest of these students in tropical medicine and by the good grounding in parasitology which they had already received in their own schools. Now that the war is over the medical schools will no doubt review their methods of teaching tropical medicine.

On the postgraduate side Dr NAPIER has asked us to look ahead, but before doing so I would recall the short war courses to medical officers of the Armed Forces, and to their American and Canadian colleagues, at the London, Liverpool, and Edinburgh schools at the R.A.F. School, Halton, and at the R.A.M. College, Millbank. At Millbank 3 000 medical officers have already attended these courses, each of which lasted a fortnight. The 100th course is now in progress.

Dr NAPIER dealt with the adequacy of courses in tropical medicine. He gave me the impression that the curriculum could easily be overburdened. The main difficulty is that at every school where tropical medicine is taught each member of the staff thinks his subject is the most important one and so tends to over-emphasize it. I so often find that the helminthologist wants to impart minute details of every single worm while the student should certainly know of each and every worm's existence, he should not spend time on learning the morphology of a parasite which is extremely rare. The only way to overcome these difficulties is a method which I have tried with some success, of asking one or two intelligent students on each course to write to me after they have spent 6 months in the tropics and tell me what in their view was the relative value of the various subjects taught.

I believe that in London there should never be a shortage of clinical material for teaching tropical medicine. As Sir PHILIP MARCOW-BALL has

said, teaching at the bedside is all important and is of greater value to the student than any systematic lecture. When a tropical centre is established, as I trust it will be, there should be no difficulty in centralizing patients for this purpose.

I should like to say finally that I entirely endorse Dr NAPIER'S plea and Dr MACDONALD'S amplification of it that this Society should take an active part in formulating a definite policy for the teaching of tropical medicine.

Dr H S STANNUS. The discussion this evening is upon the Teaching of Tropical Medicine but while the opener has touched on one aspect of the subject I think the time is ripe for taking a much wider and more progressive view inasmuch as two new factors have come to have a bearing on the problem, namely the increased facilities for rapid transport between this country and its dependencies and the awakening of our Government to the needs of our colonies.

It appears to me that the time has passed for attempting to teach the medicine of tropical countries in Great Britain because if for no other reason, clinical material will not be available.

In planning for the future—the immediate future—I would suggest that consideration be given to a scheme whereby the teaching of tropical medicine might be pushed forward to an advanced base within the enemy's country.

Briefly such a scheme would envisage the establishment of medical centres in various parts of the world—to take Africa as an example—one in East Africa, one in West Africa. At each would be established complete up-to-date hospitals both for Europeans and natives with clinical and research laboratories library and classrooms and residential college the whole organization to be under the supervision of a director and staff.

Medical officers proceeding to East African colonies having completed a shortened simplified course of instruction in laboratory methods systematic tropical medicine etc. at the tropical medical schools in this country would proceed to the East African medical centre where they might spend 6 months as house officers in both European and native hospitals and at the same time learn to correlate the large clinical experience which they would gain with their laboratory studies. They would receive training in observation, description, interpretation and presentation of the material coming under review—a side of medicine woefully neglected.

Residence in a college where talking shop must inevitably occur would assist in education as nothing else will.

Such residence would also offer an unequalled opportunity of judging the direction in which medical officers would best be employed. District medical officers could be afforded an opportunity of returning to the centre to carry out research on any problem requiring investigation, or a research team could be found for work outside the centre, etc.

The native hospital would afford opportunity for the proper training of

native hospital orderlies and subordinate medical staff. The medical, surgical, and research staff of the centre might be partly found from the Colonial Medical Service and partly from this country. An interchange between the staff of a centre and of the tropical schools in this country would benefit both. The interchange might be on a wider basis: physicians, surgeons, biochemists, etc., from this country might make visits to these centres to carry with them new knowledge while in turn they would learn something to their advantage by their sojourn abroad.

I suggest that some such scheme, while of enormous benefit to our colonies, would promote a new era in tropical medicine.

Dr H. C. Trowell. I wish to say a word about Dr NAPIER's paper and I hope that my remarks will not be misconstrued. I speak as one of those who come from a teaching centre, namely that of East Africa, and I feel that our debt to the London School and the Liverpool School and other centres in this country is very great. Indeed, we could not teach and we could not cure the diseases which we meet unless they had done their very brilliant work. But when we come to talk about tropical diseases I feel that somebody who is responsible for teaching African doctors should say a word.

There is a very great confusion of thought in this matter. We have in the first place, diseases peculiar to the tropics; in the second place, diseases commoner in the tropics than in the temperate zones; and thirdly diseases which actually occur in tropical regions but which are not due to a warmer climate. When we come to ask what diseases a fit Government official or a fit soldier is likely to contract they are usually the tropical diseases, but when we come to ask what kills the inhabitants in the tropics I doubt whether we could give the same answer.

When I come to examine the postmortem reports from any part of the world I find that the diseases specified are those which are seen in all parts of the world, but their incidence, their pattern, their characteristics, are so tremendously different that I feel we have a great deal to do in that respect in the way of clearing up the confusions which arise. When people come out to our part of the world we are hardly yet in a position to teach them all the diseases which fill most of our beds.

With regard to the main thesis of this paper, so ably presented tonight, I am in profound disagreement. It is suggested that the British undergraduate student must be given far more detailed knowledge of tropical medicine. We may differ from the Goodenough Report yet those of us who are teachers are in agreement with its main thesis. What we must teach the medical student is principles; we cannot teach detailed practice. He will stand or fall in his final examination on the application of principles as he sees them to the diseases which he meets. He will not meet much of the so-called tropical diseases, and I should regard it as entirely artificial to add an extended course on this subject for the undergraduate.

I am all in favour of adding entamoeba to biology and I trust he will be introduced to malaria and the dysenteries but to take him through the extended field of climatology and some of the other subjects set out in Dr NAPIER'S suggested syllabus seems to me entirely misplaced. Take No 11 in his suggested syllabus—herpetology. Can that be necessary? The boot may one day be on the other foot and we shall be having a Commission telling us in tropical schools to give an adequate instruction in temperate diseases!

In the tropics for example we pay very little attention to frostbite. Are we to add it to our syllabus? Are we to include scarlet fever? We pass lightly over the question of disseminated sclerosis. We teach nothing about coal miner's lung until mining has been opened up in a particular country. We can introduce the student to a few general ideas and let him work them out on the diseases which he meets. It must be our duty on the spot to work out in cases for example of cardiac disease as to why we have not yet detected subacute infective endocarditis. It is all that type of emphasis which the local school has got to give. That is why we should be so much in favour of the suggestion made by Dr STANUS that after this brief instruction at home the student should be allowed to go out to the tropics and pick up his further instruction there.

In 10 or 20 years time there will be larger medical schools in East and West Africa. After that course has been taken by the student I should hope that there would be some higher qualification whereby he could thoroughly establish himself as one competent to teach in tropical diseases.

**Sir Rikard Christophers.** I agree with most of what Dr NAPIER has said, but it seemed to me that it was not very clear what his concrete proposals were as to the points on which really important emphasis was to be laid. I am quite clear as to what I think should be done. There should be a real centre of Tropical Medicine in London. I do not mean that that should exclude Liverpool or other centres but I cannot imagine anything that would give a greater stimulus to tropical medicine than a School of Tropical Medicine in London. It is incredible that there should not be such an institution.

There is of course the London School of Hygiene and Tropical Medicine, but there tropical medicine is put in an extraordinarily subordinate place. When I address letters to that School I find myself dropping out the Tropical Medicine and calling it London School of Hygiene only. It is a very good school but we do want something more than that. Sir PHILIP MANSON BARR mentioned the time of MANSON when there was really a School of Tropical Medicine here. Not only was there a School but there was a Hospital and the spirit of Tropical Medicine. I strongly support the proposal put forward by Brig. HAMILTON FAIRLEY very ably seconded by our PRESIDENT and brought up more or less again in Dr NAPIER'S paper that there should be a combined School and Hospital of Tropical Medicine in London.

Dr Napier (in reply) After having been deflated by Sir PHILIP MASON BARR, I feel that I have been sufficiently reinflated by some of the subsequent speakers to attempt answers to some of the criticisms but as the hour is now late I cannot answer them all and must be satisfied to select certain points.

To Sir PHILIP a charge of cruelty to the long suffering medical student, I may say that I did not mean to suggest that there should be a large number of lectures on tropical subjects crowded into the student's final years or main point is that there should be a broadening of the base of the education of the medical student, so that he can profit better by any teaching on tropical subjects that may come his way later on, in the lecture theatre, the ward, or the out patient department. I have talked to several teachers who have given lectures in the short course for Army doctors and they have told me that, while some have obviously profited considerably by these lectures others have gained very little because the teaching was built on such a very frail structure of basic knowledge.

Tropical medicine is today not such a simple affair as it was in the day of Sir PATRICK MASON when the whole course of tropical medicine could be given by two or three men it is becoming much more complex. I do not think that there is any real change in our ideas on teaching tropical medicine it is just that, with our burden of greater knowledge, it is more difficult to be simple than it was in the "good old days" to which Sir PHILIP refers, and this is a fact that has to be faced with regard to all scientific teaching.

Concerning the suggested syllabus that appeared in the paper that I read last year in America, that was prepared for American conditions and is keeping with the current ideas in that country that an adequate course of tropical medicine for postgraduate students should last 9 months and consist about a thousand hours of instruction. It was not meant for this country where, rightly or wrongly shorter courses are usually given, and I would not presume to suggest that the teaching in the schools in this country should be altered to conform with that syllabus. However Dr MACDONALD has pointed out to me that all the subjects that I have mentioned are actually touched upon in the course at the London School, with the exception of economics.

Professor GORDON protested against my emphasis on London for a tropical diseases centre. I did not mean that London should be the only place where attention should be given to the subject of tropical medicine but it is a fact that, for the time being at any rate, London is not only the capital but the largest medical centre in the British Commonwealth, and it does seem incongruous that there should not be a first-class tropical medicine centre here, though of course not to the exclusion of other centres.

With reference to Professor GORDON's last point, on the need for sending only properly trained doctors out to the tropics it is true that I made no reference to this subject in my paper to-day. However I elaborated it at some length in my American paper to which he referred and in my desire to

keep my paper short and as far as possible to avoid repetition I assumed that in Great Britain it would be unnecessary to emphasize this point. I am extremely sorry to hear that my assumption was not correct, and I am glad that Professor GORDON has rectified the omission.

I am in entire agreement with Dr MACDONALD's amplification of my suggestion, and I strongly support his recommendation that there should be a standing policy committee of this society.

Colonel VERE HODGE is perfectly right. It will certainly be necessary in the immediate future to introduce a number of short courses as a stop-gap to meet the urgent demand for some teaching in tropical medicine.

Colonel DREW mentioned that there were lectures on tropical medicine in all the medical schools in London before the war. This is true, and my complaint was that the war was made an excuse for stopping these rather than a reason for developing and extending them, as was done in the United States. I have heard from many directions that his special lectures to medical students in London were very much appreciated, but they were voluntary lectures and, as most of the schools were decentralized, only a comparatively small proportion of the students were able to attend them. I know that questions on tropical diseases have been set in several final examinations recently and I am not suggesting that there should be one in every final examination paper but only that the student should be taught to accept the idea that there may be such a question.

Regarding Dr TROWELL's criticism, I believe that our views are not so divergent as he seems to think. He has talked mainly of the teaching of medicine to the natives of a tropical country (who will incidentally mostly remain and practise in their country of origin) whereas I was considering the teaching of the science of medicine to men from among whom many will later go to practise in tropical countries and others will frequently see examples of tropical diseases in their own country.

The syllabus that I suggested, which has so alarmed Dr TROWELL, was naturally not for the undergraduate student but for a postgraduate course, and I was under the impression that the word "herpetology" which he selected for special scorn, meant the study of snakes, not an inappropriate subject for a tropical course.

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Colonel H E Shortt (contribution to discussion submitted after the meeting) I have listened with interest to the opening address tonight by Dr NAPIER and to the subsequent discussion by those who are actively engaged in the teaching of tropical medicine. I think most of us would agree with the majority of the opinions expressed on the most advantageous methods of teaching this subject and, in spite of somewhat differing viewpoints the opinions all coincide on the most important points.



Dr NAPIER, I think, lays undue stress on the view that tropical medicine should in no way be differentiated from general medicine, and that the medical practitioner in this country has left his education incomplete unless he has a good working knowledge of tropical diseases as well as those he is most likely to meet with in this country. I cannot agree with this point of view because I think tropical medicine is to a large extent an entity apart from general medicine. By this I do not mean that the two can be separated into watertight compartments and this is more especially the case from the point of view of the practitioner in the tropics.

The medical practitioner in this country has got along very well in the past with no more than a sketchy knowledge of tropical diseases, and since the medical curriculum is probably already overloaded he will have to do so in the future relying when necessary on the advice of experts in tropical medicine. The practitioner in the tropics on the other hand, is in a somewhat different position, and he would be extremely ill equipped were his knowledge confined to tropical diseases only. He must be a good general physician or surgeon, on whose knowledge has been grafted the extra knowledge and skill required for dealing with the special diseases he will meet in a tropical environment. In other words, the study of tropical medicine is as much a speciality as medicine, gynaecology, diseases of children, and so on. Such a knowledge of tropical medicine is not acquired except as the result of more or less prolonged residence in the tropics and a conscientious use of the material available there.

From this it follows that even if tropical medicine formed part of the medical curriculum in this country the opportunities to see the actual cases of the various tropical diseases would be too limited in the case of most practitioners, and they would still have to fall back on the advice of the expert. I have seen in my experience too much of the results of faulty or insufficient knowledge both in the diagnosis and the treatment of tropical diseases, to leave me in any doubt as to the necessity of looking upon tropical medicine as a special branch of medicine.

Having dealt with this principle I should like to offer a few remarks as to what I consider would be the best methods of attaining our object, which I envisage as providing the means first for training adequately postgraduate students who wish to take up the study of medicine in the tropics, and second the provision of facilities for research to those who are engaged on special studies on some aspect of tropical medicine. The first essential to attain these objects and one which is so important that all others are minor matters in comparison, is the provision of a Tropical Diseases Hospital, worthy of the capital of the British Empire. While such a hospital, or if the term is preferred, Tropical Diseases Centre, was desirable in the past, it is absolutely essential now, in view of the changed conditions in the British Empire as part of a world-wide change in conditions which has come about in the last 20 years. The special need for such a centre depends on two or three factors which were less important

in the past. In the first place during a 6-year period there has been little or no full-scale teaching of tropical medicine such as was provided before the war at three centres in the United Kingdom. Almost complete cessation of recruitment to the colonial services during the war has resulted in much leeway to be made up in the provision of medical officers for the colonies and protectorates. In the second place, before the war there was already among many foreign nations an increasing desire for the training of a number of their medical men in tropical medicine on the latest lines. This desire will almost certainly be reawakened and intensified as conditions of peace are re-established in countries which have any dealings, either within their own borders or by commerce with tropical countries. In the third place, tropical diseases have already acquired an importance which is likely to increase in countries far removed from the tropics; this is due to the cutting down of the distance both in space and in time of the tropics from temperate countries by modern means of transport. Whatever the quarantine precautions taken, it will not be possible to prevent the entry into any country of persons infected with diseases in other countries so that the knowledge of these diseases and of the methods to prevent their entry and to deal with them if they do enter will be more than ever necessary and will require the training of the necessary personnel for such purposes.

All this leads us back to the absolute necessity for the immediate provision of tropical diseases centres working in close collaboration with the present schools of tropical medicine for the adequate training of the men who will carry out the essential measures which will be necessary in the near future.

It is easy to talk of the establishment of a tropical diseases hospital, and school, in more or less vague terms, but if the scheme is to be rapidly translated into fact one should know beforehand exactly what would be required in the way of staff and the scale on which this should be provided if the working of the scheme is to be efficient. So far as the hospital or treatment centre is concerned this would be staffed in general along the lines of any modern hospital and the differences would only be in detail. The hospital would have to be within easy reach of the teaching institution. There is no question that the ideal would be to build *de novo* a new hospital specially for the purpose but as this may be unobtainable in the immediate future consideration should be given to the acquisition and conversion of a suitable building as a temporary measure into a hospital. On this point I think there would be no disagreement. So far as the teaching institution is concerned there is more room for differences in outlook and there is no reason why there should not be individuality in the pattern of these providing there is uniformity in the end aimed at which is the provision of up-to-date and adequate teaching on all aspects of medicine in the wider sense concerned with the tropics.

My own knowledge is limited to that part of the training concerned with the teaching of medical zoology which on the clinical side forms the greatest part of the background of knowledge to be acquired by the student of tropical diseases.

To leave the question of a hospital, and to come to the requirements for the teaching in medical parasitology in the wide sense, that is including the subjects of helminthology protozoology and entomology the facilities present before the damage done due to war were probably adequate as regards accommodation, and should be so again when this damage has been fully repaired. The same perhaps cannot be said in regard to staff and certainly not at the present moment in speaking of the three subjects I have already mentioned. I would consider the following suggestions with regard to the staffing of the departments as a minimum (1) For each subject there should be a head of the particular department with an assistant, that is a professor with a reader or a lecturer. The object of this would be both to allow the carrying on of research and teaching simultaneously in the department, and to enable one of the senior members at any time to proceed abroad in order to carry out a line of research or enquiry which could not be done in this country. Another reason for the providing of a full staff in each department is the fact that special enquiries requiring the collaboration of helminthologists and entomologists and protozoologists could be undertaken by the formation of a special unit combining workers in these two or more subjects. This method of special units formed to investigate specific problems has been very successfully employed in the past in India, and could well be copied. This sort of team work would also serve to integrate the activities of the three departments in a way which would be useful to each, and which could not be attained easily by other means.

What I have said has necessarily been a mere outline but serves to indicate the way in which I think the teaching of tropical medicine could best be carried out. There has been so much delay already over the question of the provision of a Tropical Diseases Hospital or Centre that I feel some more concrete step is now needed than any taken up to the present. The matter is one of vital importance to the Empire and I think should now be taken up at the highest level by the departments of Government most closely concerned, that is, the Colonial Office, Dominions Office and the India Office.

To implement a scheme as outlined above would require a large non-recurring grant for the provision of buildings or the acquisition of already existing buildings as a temporary measure and an annual recurring grant for salaries and equipment and general maintenance of the organization. Without going into detail it is not possible to give any idea of what sums would be required, but I consider that a small *ad hoc* body representing the three Government departments concerned, and the three institutions at present concerned with the teaching of tropical medicine, should be set up immediately to report in detail on any scheme agreed to and that this body be given a time limit in which to make their recommendations.

## COMMUNICATIONS

### AUTOANTIGENS AND AUTOANTIBODIES IN THE PATHOGENESIS OF DISEASE WITH SPECIAL REFERENCE TO BLACKWATER FEVER.

BY

JAMES GEAR Major  
*Medical Laboratory Service S.A.M.C*

In the course of another investigation undertaken in the Laboratories of the International Health Division of the Rockefeller Foundation in 1942 it was shown that the inoculation of an emulsion of fresh normal liver obtained from a healthy rhesus monkey into either a normal monkey or into one previously protected by vaccination against yellow fever failed to elicit any antibody response. On the other hand it was found that an emulsion prepared from the liver of a rhesus monkey that had died of yellow fever was antigenic when inoculated into a rhesus monkey previously protected by vaccination against yellow fever. This latter monkey developed antibodies which could be demonstrated by the precipitin test not only against an emulsion of yellow fever infected liver but also against an emulsion of normal liver. It appeared therefore, that the presence of virus in liver cells acted as a *schlepper* for normal liver substance which thus became antigenic when introduced into a monkey of the same species.

Assuming that a substance which was homologously antigenic would also be autoantigenic and assuming that antibodies are produced by the reticulo-endothelial system, this finding may be diagrammatically presented thus —

Liver cell + virus	= Autoantigen.
Autoantigen + R.E. cell system	= Autoantibody
Autoantigen + autoantibody	= Reaction causing degeneration of affected cell.

This interesting observation as well as indicating a possible sequence of events in the evolution of the pathological process of yellow fever suggested a hypothesis of the pathogenesis of some disease conditions which may be summarized thus —

Tissue cell + virus	} = Autoantigen
or + bacterial toxin	
or + chemical	
or + intracellular parasite	

Autoantigen + R.E. cell system = Autoantibody

Autoantibody + tissue cell

affected or sometimes + normal tissue cell

= Sensitized cell.

Sensitized tissue cell + complement = Degeneration or lysis of affected cell.

This hypothesis has already been advanced to explain such obscure conditions as post vaccinal encephalitis and other post infective encephalides (SCHWENTKER and RIVERS 1934) and glomerular nephritis (SMADEL, 1936 SCHWENTKER and COMPTON, 1939). It is to put forward and discuss a similar hypothesis of the pathogenesis of blackwater fever that is the main purpose of this paper.

There is now general agreement that blackwater fever is related to malaria and is especially liable to affect those who have suffered from repeated attacks which have been inadequately treated with quinine. It was once considered that blackwater fever was associated only with malignant tertian malaria (THOMSON 1924) but later observations (FOY 1938) have shown that in Salonika almost as great a proportion of cases are associated with *Plasmodium vivax* (33 per cent.) as with *P. falciparum* (47 per cent.) while mixed infections were noted in 14 per cent. of cases.

Although many theories have been advanced to explain the acute intravascular haemolysis which is the fundamental event in an attack of blackwater fever the cause to summarize current literature and textbooks, remains a mystery. It has often been suggested, however, that a haemolysin or an anaphylactic sensitizing substance is responsible for the haemolysis, but so far no adequate explanation for their development has been put forward. To quote *Stuart's Tropical Diseases* (STRONG 1942) "A scientific explanation of the mechanism by which haemolysis is brought about is not yet possible. It is conceivable that a haemolysin may be free in the blood stream or bound to certain cells, but it is not clear what suddenly frees it or precipitates its action."

However it appears that in the hypothesis enunciated above a reasonable explanation is found. According to this hypothesis the development of haemolysis may be accounted for by the fact that a red cell infected with a malarial parasite possibly only after treatment with an antimalarial drug, especially quinine may become antigenic. The sequence of events may be formulated as follows —

Red cell + malarial parasite

or

Red cell + malarial parasite + antimalarial drug

or

Red cell + antimalarial drug

Autoantigen + R.E. cell system = Autoantibody (haemolysin).

Red cell + haemolysin = Sensitized red cell.

Sensitized cell + complement = Lysis of red cell.

There is a vast collection of facts relating to blackwater fever, and these have often been analyzed and considered in the past. However, it is now desirable to re-examine and to discuss them as well as several recent observations in relation to this hypothesis of its pathogenesis. It will become apparent in this discussion that these facts are all in keeping with, or are rationally explained, by this hypothesis.

There is little direct evidence of the presence of a haemolysin in the blood of cases of blackwater fever. To quote THOMSON (1924) 'Attempts to demonstrate an autohaemolysin in the serum of blackwater fever during the attack of haemoglobinuria have failed or indefinite results have been obtained.'

'We were quite unable to detect any haemolysin in the serum of blackwater fever at any period of the disease but as we have pointed out this by no means proves that a serum haemolysin is not present.'

Indeed, THOMSON states "There are strong grounds for suspecting that the condition is brought about by a specific serum haemolysin elaborated from the prolonged action of *Plasmodium falciparum* on the red blood corpuscles. He suggests that, "it is an altered chemical change which produces the so-called brassy corpuscles in malignant tertian. Corpuscles so altered act as foreign bodies and are thus capable of producing a specific haemolytic amboceptor which can act in the presence of complement on corpuscles so altered and not on normal red cells.'

ROSS (1932), although stating that there is no doubt as to the reality of the appearance of the altered corpuscles that THOMSON describes, states that attempts to demonstrate the presence of such altered cells were very seldom successful during his investigation, and therefore considers the hypothesis regarding the role of such cells as open to criticism.

FAINLEY and BROMFIELD (1934) came to the conclusion that the intravascular haemolysis of blackwater fever was caused by a haemolytic agent arising in chronic subtertian malaria as a result of a metabolic breakdown precipitated by quinine or plasmoquine, or possibly by other factors such as chill or exhaustion. They considered their findings were against either the action of an immunological haemolysin or a direct drug effect on the corpuscles.

Although the direct evidence is so unconvincing there is considerable indirect evidence that an antibody acting on red cells is present in cases of blackwater fever. This will now be considered.

It has repeatedly been observed that red cells from cases of blackwater fever show a tendency to autoagglutination. This in itself is suggestive of the presence of antibodies but by itself is not conclusive for autoagglutination of the red cells has been observed in many conditions that are not associated with acute intravascular haemolysis. For example, autoagglutination of the red cells is often observed in the blood of cases of human trypanosomiasis. However even in this condition erythrophagocytosis has been noted, a phenomenon which suggests the presence of red cell opsonins.

Erythrophagocytosis has also been noted in cases of blackwater fever. THOMSON (1924) in his observations on eight fatal cases of blackwater fever, noted that there was evidence of endothelial activity and the endothelial cells showed active phagocytosis of the red cells. YORKE, MURGATROYD and ORR (1929) note that phagocytes containing red cells are frequently seen both in films of the peripheral blood and of the splenic pulp. ROSS (1932) also reported that smears from the spleen of cases coming to autopsy showed the presence of phagocytosed red cells and in several instances the phenomenon was also present in films of the peripheral blood. The engulfed red cells always appeared to retain their haemoglobin. For that reason he considered that the appearance indicated the presence of an increased erythrophagocytosis in the disease. These authors all conclude that its importance in the haemolytic process is probably small, but that it may account for part of the reduction that occurs in the red cell count. However its occurrence suggests the presence of red cell antibodies.

Autoagglutination of the red cells was clearly seen in the two most recent cases of blackwater fever admitted to the Johannesburg Hospital. Both these cases in their early stages also showed a tendency for the red cells to be microcytic and hyperchromic an observation in turn indicating that there was a tendency to spherocytosis.

Spherocytosis in cases of blackwater fever was noted as long ago as 1906 by CHRISTOPHERS and BENTLEY (1908). THOMSON (1924) confirmed this finding noting that in stained preparations these small spherical erythrocytes assumed much darker red than the other red cells when stained with Leishman. More recently in a case of blackwater fever admitted to the Children's Hospital, Johannesburg FOY and KONDI (1943) studied this question in detail and found that cells from a case of blackwater fever were more spherocytic than normal but less so than those of haemolytic jaundice. Spherocytosis of the red cells, of course, is one of the characteristic findings in familial haemolytic jaundice. DAMESHEK and SCHWARTZ (1938) suggest that this condition is caused by an autohaemolysin and has shown that one of the earliest demonstrable effects of a haemolysin acting on a red cell is for the affected cell to become spherocytic. Accordingly the occurrence of spherocytosis in blackwater fever favours the hypothesis that this condition is caused by a haemolysin.

Two other important observations that suggest the presence of haemolysin similar in action to artificially produced antibodies have been made by FOY and KONDI.

FOY, KONDI and MOUNGIDIS (1941) showed that red cells transfused into a haemolytic case of blackwater fever from three different donors underwent haemolysis just as readily as did the blackwater fever patient's own cells. This was interpreted as indicating that there is some circulating haemolysin that destroys all red cells that come into contact with it, irrespective of their origin. This finding is what would be anticipated if an immunological haemolysin is

concerned, thus donors cells on transference to the recipient would become sensitized by the haemolysin present in the recipient's circulation. The cells are thus rendered susceptible to the action of complement and so to haemolysis.

Later, Foy *et al* (1945) showed that the red cells from a case of black water fever when transfused into a normal individual were haemolyzed as rapidly as those remaining in the patient. This observation is also what would be anticipated if an immunological haemolysin were concerned, for the patient's red cells are already sensitized by the autohaemolysin so that, even if transferred to a healthy person they remain susceptible to the action of complement and are haemolyzed.

That an antigen antibody reaction is probably concerned in the pathogenesis of blackwater fever is also suggested by a consideration of other conditions characterized by intravascular haemolysis. Two of the best known of these are paroxysmal haemoglobinuria and incompatible blood transfusion. Both are known to be due to red cell antibodies which can be demonstrated *in vitro*.

A condition analogous to blackwater fever can be brought about experimentally by the injection of an artificially prepared haemolytic serum into experimental animals. CHRISTOPHERS and BENTLEY (1908) and DUDGEON (1920) who investigated the effects of haemolytic sera on animals showed that the pathological appearances were the same as produced in blackwater fever. THOMSON (1924) remarks there is so marked a similarity between the action of a true specific serum haemolysin and an attack of haemoglobinuric fever that there can be little doubt that the mechanism is the same and if we can produce such a condition by continual attacks of malaria the problem is solved.

It is known that blackwater fever rarely affects an individual who has been resident in a malarious area less than 6 months, but thereafter the chances of developing an attack increase with length of residence. The greatest number of cases occur in individuals who have lived for from 1 to 3 years in an area where malaria is hyperendemic, and who during this time have usually suffered from repeated attacks of malaria often inadequately treated with quinine.

This time lag between the first infection with malaria and the development of blackwater fever in itself suggests a process of sensitization. As the time taken for sensitization exceeds 6 months it is reasonable to conclude that the antigenic value of the hypothetical autoantigen is relatively low so that repeated attacks of malaria are necessary before a state of sensitivity develops. It may well be that, in addition to malaria treatment by antimalarial drugs is a necessary factor for the development of this state.

The evidence discussed above may now be summarized —

- 1 Autoagglutination and spherocytosis of the red cells in cases of black water fever have been observed.



2. Erythrophagocytosis is commonly seen in smears made from the spleen and occasionally in films of peripheral blood.

3. Red cells from healthy donors haemolyze as rapidly in a case of blackwater fever as the patient's own cells.

Red cells when transfused into a normal person from a case of blackwater fever haemolyze as rapidly as the red cells remaining in the patient.

4. Blackwater fever is closely simulated by paroxysmal haemoglobinuria and incompatible blood transfusion, conditions both caused by known and demonstrable anti red cell antibodies and by the effects of artificially produced haemolytic sera in experimental animals.

5. There is usually a lag period between the first attack of malaria and the onset of blackwater fever. This time interval suggests a process of sensitization.

All these facts are readily explained on the assumption that an autohaemolysin is concerned in the pathogenesis of blackwater fever. Other hypotheses put forward cannot satisfactorily account for them. Indeed, it may be concluded that blackwater fever is almost certainly caused by an autohaemolysin.

One of the most puzzling features of blackwater fever has always been its sudden and dramatic onset. Sir PATRICK MANSON put this puzzle in his question to Dr THOMSON in 1922. "Malaria is undoubtedly one of the factors but what pulls the trigger?"

In *Stitt's Tropical Diseases* (STRONG 1942) it is stated that it is conceivable that a haemolysin may be free in the blood stream or bound in certain cells, but it is not clear what suddenly frees it or precipitates its action.

It is considered that the hypothesis put forward in this paper also accounts for the sudden onset of blackwater fever. In this connection it is relevant to discuss the site of formation of the haemolysin. There has been a considerable amount of experimental work on the site of formation of antibodies, and an extensive literature dealing with this subject has accumulated, but it is not intended to review it here. Suffice it for the present to note that much evidence has been presented favouring the view that antibodies are produced by the reticulo-endothelial system of cells. The spleen is the largest depot of this system of cells in the body. Several observations have been made which show that the spleen is intimately connected with serum immune bodies. It is known that the removal of the spleen seriously undermines the resistance of rats to an infection with *Bartonella muris*. An infection which previously was easily tolerated, after removal of the spleen becomes rapidly fatal.

In the field of human medicine removal of the spleen is known to benefit the clinical condition of cases of familial haemolytic anaemia, a condition which is possibly caused by autohaemolysins (DAMESHEK and SCHWARTZ, 1936). Splenectomy also benefits many cases of chronic thrombocytopenic purpura and some cases of chronic leucopenia. Incidentally it is considered very likely

that these two conditions are also caused by autoantibodies, and will be considered from this point of view of this hypothesis in more detail at a later stage.

It was noted, incidentally in a paper on cases of Rhodesian sleeping sickness (GEAR and DE MEILLOV, 1939) that smears made from the spleen of experimentally infected animals showed few intact trypanosomes, although degenerating forms and remnants of degenerated forms, were numerous. In contrast, smears made from all the other organs including the lymph glands showed numerous intact trypanosomes. It seemed from this observation that the spleen is the principal site of the formation of trypanosomocidal antibodies. It is reasonable to expect that it is also the principal site of formation of other circulating antibodies. It is likely then, that haemolysins responsible for the acute intravascular haemolysis of blackwater fever would also be formed principally in the spleen. It would be expected, then, that when a person had become sensitized, any factors causing a sudden contraction of the spleen so expressing its contained haemolysins into the general circulation, would result in an attack of blackwater fever.

On referring to the known precipitating causes of blackwater fever it is found that they are usually given in this order quinine chill, exertion and violent emotion. These the chief exciting causes of this fever have one effect in common they cause contraction of the spleen.

Thus it becomes clear why the onset of an attack of blackwater fever is so sudden and dramatic. It is only when a sudden contraction of the spleen occurs that sufficient haemolysin is liberated to cause intravascular haemolysis. At other times it seems probable that the haemolysin is mopped up as it is formed and the number of red cells so sensitized is not too large to be removed by the reticulo-endothelial cells before intravascular haemolysis takes place. In that case one would expect that cases of subacute or chronic haemolytic anaemia, not associated with intravascular haemolysis would occur. That such cases do occur has been made clear by FAIRLEY BROMFIELD FOY and KOVDI (1938) who described cases of haemolytic anaemia associated with chronic malaria in Macedonia. It is very interesting to note that these cases occurred in Macedonia, a region where more cases of blackwater fever are seen than anywhere else in the world. It seems possible, then, that these cases are more or less benign manifestations of the process which in more violent form results in blackwater fever.

Several writers on blackwater fever have considered that in some way or other the spleen is involved in the pathogenesis of blackwater fever. BLACKLOCK and MACDONALD (1928) believed that the sudden massive haemolysis is occasioned by the over production of sarcolactic acid a normal constituent of the blood and tissues. This is caused by anaemia particularly in the spleen where the adherence of malaria infected red cells to each other and to the vascular endothelium interferes with the circulation. They point out that the agencies chill exertion quinine which precipitate an attack, all cause

contraction of the spleen, and this they consider accentuates the existing anaemia so that lactic acid formation and accumulation increase to haemolytic concentrations and leakage of haemoglobin into the portal circulation results. This leakage becomes increasingly apparent as circulation through the spleen becomes re-established leading to haemoglobinaemia and haemoglobinuria. Ross (1932) studied cases from the point of view of this hypothesis and concluded that the theory on which it was founded was fallacious.

FAIRLEY (1940) suggests that a perversion of the activity of the reticulo-endothelial system occurs as a result of chronic malaria, which induces a considerable hypertrophy of this system in general and of the spleen in particular. He suggests that an escape into the blood stream from a pathological reticulo-endothelial system of the intracellular lytic enzyme, normally responsible for the destruction of effete red cells occurs and is responsible for the intravascular haemolysis.

VINT (1941) puts forward a hypothesis of the causation of blackwater fever based on experiments of FAIRBAIRN (1939), who showed that under certain conditions red cells undergo a change he called stabilization. VINT suggests that a similar change occurring in the splenic vein is responsible for the lability of red cells in cases of blackwater fever to haemolyze.

FOY and KORDI (1943), who found that the red cell fragility to lysolecithin was increased in cases of blackwater fever state. The enlargement of the spleen in so many of these haemolytic conditions is regarded by many as of considerable significance. The production of lysolecithin as a result of the separation of the cells and plasma in this organ has led to the suggestion that this powerfully haemolytic substance may play a part in the haemolysis of certain of these conditions.

Although the hypotheses put forward by these authors differ often very considerably in their basis they all bring forward evidence that in some way or other the spleen is intimately concerned in the pathogenesis of blackwater fever. It is considered that the role assigned to the spleen in the hypothesis here put forward accords with the observed facts more satisfactorily than the other hypotheses of the causation of the condition. Most of these hypotheses do not explain what might be called the specific nature of blackwater fever, a specificity that is clearly explained by the assumption that a biological haemolysin is concerned.

It is now opportune to suggest the probable sequence of events leading up to an attack of blackwater fever.

Red cells infected with malarial parasites, possibly only after treatment with an antimalarial drug or red cells altered by or combined with an antimalarial drug become autoantigenic, and in response to this autoantigen an antibody or haemolysin is produced by the reticulo-endothelial system, particularly by the spleen. The titre of this haemolysin is boosted by each repeat attack of malaria. When the circulation of the blood through the spleen is

free, this haemolysin is mopped up by the red cells and these sensitized red cells are removed by the reticulo-endothelial cells as they are sensitized and before a demonstrable intravascular haemolysis occurs. However, when the circulation through the spleen is impeded, and the spleen becomes congested, as it does in an attack of malaria which also provides a secondary stimulus to boost the titre, this haemolysin accumulates. Factors which cause a sudden contraction of the spleen, such as the administration of quinine chill and exertion, now would suddenly express into the general circulation sufficient haemolysin to sensitize a large number of red cells and their haemolysis intravascularly would result in haemoglobinaemia and haemoglobinuria followed by the other signs and symptoms of blackwater fever.

The acceptance of this hypothesis of the pathogenesis of blackwater fever may be of practical value. If the haemolysin is produced by the spleen and expressed from it by conditions which cause that organ to contract it is obvious that it is desirable to prevent such contraction especially sudden contraction. Measures to ensure this would be —

Placing the patient at absolute rest in bed lying flat

Placing his mind at rest

Avoidance of drugs which would cause smooth muscle contraction

It would also be desirable if such were available to administer a drug which would prevent the union of antibody and antigen.

The commonly accepted method of treatment of cases of blackwater fever aims at achieving these objects: thus to quote *Stitt's Tropical Diseases* (STRONG 1942) Absolute rest in bed, avoidance of chilling and good nursing are prime considerations in treatment. Recently BURKITT (1943) wrote that when treating cases of blackwater fever in East Africa he was struck by the great restlessness of mind and body characteristic of that disease and to counteract this gave 8 to 10 grains of sodium luminal intramuscularly. In eight cases so treated in the evening all traces of blackwater had disappeared by the following morning. He also reported that Dr J. K. GREGORY of Nairobi has had a series of thirty cases all treated by intravenous injection of 15 grains of phenobarbitone and all cured at once. He remarks that he has used the unscientific expression cured at once because it gives the picture exactly. A reasonable explanation of the success of this sedative treatment has been given in this discussion.

The advisability of the administration of quinine to cases of blackwater fever is still a vexed question. Arguing on the basis of the hypothesis here put forward it would seem that, once the spleen had contracted and expressed its haemolysin there would be little danger in treatment with quinine unless it was withheld for a sufficient time to once again allow a considerable amount of haemolysin to dam up in the spleen. However as then it would be a real danger it would seem that treatment with quinine should not be given for the concomitant malaria unless mepracrine is not available. Although the

evidence is not altogether convincing it is generally accepted that the regular taking of prophylactic quinine decreases the liability to blackwater fever. It is known that the adequate treatment of malaria by quinine likewise diminishes the chances of blackwater fever. Yet the irregular taking of prophylactic quinine, and the inadequate treatment of malaria by quinine, are recognized as most important factors in rendering a patient prone to blackwater fever. A reason for even this paradoxical state of affairs emerges from this discussion. It has already been noted that the antigenic value of the autoantigen is probably low and that it usually requires a prolonged exposure to its action before a state of sensitivity develops. Such a prolonged action would be ensured by irregular prophylactic quinine, and by inadequate treatment, both of which would allow a latent infection to persist and to progress, but which would also prevent such an infection from frankly asserting itself thereby lessening the body's natural development of immunity. Also because of the inadequate treatment relapses are frequent and provide the secondary stimuli which result in boosting the titre of an autohaemolysin.

Accordingly from the point of view of prophylaxis it is important that, if a person is taking prophylactic quinine, he should do so regularly but more important still should he contract malaria it is essential that his treatment should be thorough.

It seems that thorough treatment, by eliminating the potential autoantigen, guards against the development of the autohaemolysin. In cases of chronic malaria that have already suffered from blackwater fever it is essential that they should be thoroughly treated, because such thorough treatment eliminates the autoantigen and it appears that once this is eliminated the titre of the autohaemolysin rapidly diminishes. This was clearly demonstrated in a case of recurrent blackwater fever induced by quinine described by FARRIS and MURGATROYD (1940). The capacity of quinine to produce blackwater fever appeared in this case to be directly or indirectly related to persisting malarial infection, which was associated with a demonstrably enlarged spleen. Following effective treatment by atabrin and disappearance of the splenomegaly the administration of quinine was no longer followed by haemoglobinuria.

Regarding the prevention of the union of antigen and antibody it is relevant to note that such properties have been ascribed to acetyl salicylic acid. This therapeutic effect no doubt accounts for its great value in relieving the symptoms of acute rheumatic fever a condition which it seems very likely will also be found to be caused by an autoantigen-antibody reaction taking place in tissues rendered autoantigenic by union with haemolytic streptococcal toxin. As far as can be ascertained aspirin has not been credited with any therapeutic effect in cases of blackwater fever. It would be interesting to know the results of a trial of this drug over a large series of cases though no striking effect could be anticipated, because once haemoglobinuria has occurred, the

process has in most cases expended itself. However its administration may be of value in the remittent type of case.

The other lines of treatment such as blood transfusion for combating the effects of the intravascular haemolysis although they are of the utmost importance and often life-saving will not be considered in detail as they do not immediately concern our theme. It is however pertinent to discuss briefly other conditions characterized by intravascular haemolysis noting whether any feature of them has any bearing on this hypothesis. Of these conditions attention has already been drawn to the effects of an incompatible blood transfusion and to paroxysmal haemoglobinuria, both of which are caused by demonstrable antibodies. Of other conditions one of the most common is acute haemolytic anaemia following the administration of certain drugs. Some of these such as phenylhydrazine act directly on the corpuscles. In others the reaction appears to result from an undue sensitivity. In recent years the drugs most commonly concerned in the latter group are those of the sulpha group. We have seen two cases of acute haemolytic anaemia following the administration of sulphapyridine. These will not now be described in detail suffice it to note that in each case towards the end of a course of treatment, acute haemolytic anaemia suddenly developed characterized by intravascular haemolysis as shown by haemoglobinaemia and haemoglobinuria.

It is recognized that these accidents are rare and usually they are attributed to an idiosyncrasy of the patient. This idiosyncrasy is usually considered to be of an allergic nature. In the hypothesis here discussed a rational explanation for their development is found which can be formulated as follows —

Red cell + sulpha compound or derivative	= Autoantigen
Red cell + antibody (haemolysin)	= Sensitized red cell
Sensitized red cell + complement	= Haemolysis.

In the cases noted above one was fatal, dying of uraemia following anuria, the other rapidly recovered after withdrawing the drug. As the condition developed soon after exposure to the drug it would appear that red cells linked to the sulpha drugs have a high antigenic value which accounts for the relatively rapid development of haemolysin, but as this antibody unlike the haemolysin of blackwater fever apparently acted only on red cells that had come into contact with the drug recovery was rapid and complete.

We have also seen two cases of malaria in their primary attack, which towards the end of a course of treatment with atebnin suddenly developed an acute haemolytic anaemia, characterized by haemoglobinaemia and haemoglobinuria. Although these cases being associated with malaria were considered to be cases of true blackwater fever it is our opinion that they were more directly comparable with the type of haemolytic anaemia in the two cases noted above resulting from an acquired sensitivity to the drug. It seems too that the cases of quinine haemoglobinuria of which many have been recorded also belong

to this group. The acute haemolytic anaemia of favism likewise appears to belong to this group.

Possibly similar in pathogenesis were two cases of aplastic anaemia, recently seen in the Johannesburg Hospital. One followed a full course of treatment with sulphapyridine for pneumonia, but it is of interest to note that the signs of aplastic anaemia did not become evident until 3 months later. The other similarly followed a course of treatment with sulphapyridine and sulphonamide for tonsillitis.

It is also of interest to note that we have under observation one case of chronic agranulocytosis that first manifested symptoms 10 months after the second full course of treatment with sulphapyridine for gonorrhoea. The blood film of this case in which the leucocyte count was usually in the neighborhood of 2,000 was characterized by a considerable proportion ( $\pm 20$  per cent.) of disintegrating neutrophil leucocytes. A similar blood picture can be artificially produced in experimental animals by the injection of an antileucocyte serum.

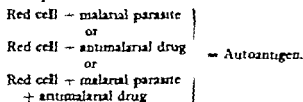
Another apparently analogous case at present under observation is that of a patient who following a full course of treatment for pneumonia with sulphapyridine developed thrombocytopenia. This first manifested itself 1 month after recovery from pneumonia and now has persisted for 2 years. It is relevant to note that thrombocytopenia can be produced experimentally by the injection of antiplatelet serum. These interesting cases will be described and discussed in detail in another paper. They are mentioned now because it seems that a rational explanation of their pathogenesis is to be found in the hypothesis set out in this paper.

The more fundamental problems of the constitution of antibodies and of complement have not been discussed. It seems probable that such a study may show that it is possible to correlate some of the, at present, apparently diverse theories of the pathogenesis of blackwater fever.

## SUMMARY

A hypothesis of the pathogenesis of blackwater fever suggested by the observation that liver emulsion from a rhesus monkey dead of yellow fever stimulates the formation of antiliver antibodies when injected into another rhesus monkey is put forward and discussed.

This hypothesis may be formulated thus —



Autoantigen + R.E. cell system = Autoantibody haemolysin  
 Red cell + haemolysin = Sensitized red cell  
 Sensitized red cell + complement = Haemolysis

It is noted that, in cases of blackwater fever the red cells show auto-agglutination and spherocytosis and that erythrophagocytosis is commonly seen in smears of the spleen and, occasionally in films of peripheral blood. Further, it has been observed, red cells from healthy donors haemolyze as rapidly in a case of blackwater fever as the patient's own cells and on the other hand, red cells from a case of blackwater fever transfused into a healthy recipient, haemolyze as rapidly as the red cells remaining in the patient.

There is usually a considerable interval between the first attack of malaria and the onset of blackwater fever during which the patient suffers from repeated attacks of malaria, often inadequately treated with quinine.

It is noted, too, that blackwater fever is closely simulated by the effects of an incompatible blood transfusion by paroxysmal haemoglobinuria and by the effects of the injection of an artificially produced haemolytic serum into experimental animals.

All these observations are readily explained on the assumption that a biological autohaemolysin is concerned in the pathogenesis of blackwater fever. Other hypotheses put forward cannot satisfactorily account for them especially for the specific association of blackwater fever with malaria. It is concluded, therefore, that an autohaemolysin is almost certainly concerned in the production of blackwater fever.

The site of formation of this autohaemolysin is discussed, and it is considered that it is mostly produced in the spleen.

The boosting of the titre of the autohaemolysin resulting from repeated attacks of malaria often inadequately treated and factors producing a sudden contraction of a congested spleen in which autohaemolysin has accumulated are considered likely to precipitate an attack and to account for the sudden dramatic onset of blackwater fever.

The treatment of the condition in the light of this hypothesis is discussed and it is considered important to prevent such sudden contractions of the spleen. It is also important, in order to avoid the development of a state of sensitivity to treat malarial infections adequately.

Other conditions characterized by intravascular haemolysis are briefly discussed. It is noted that apart from incompatible blood transfusion, and paroxysmal haemoglobinuria which are known to be caused by biological antibodies such conditions as the acute haemolytic anaemias following certain drugs and of favism can also be reasonably explained.

Cases of aplastic anaemia of agranulocytosis and of thrombocytopenia are briefly described and it is noted that an explanation for their development is possibly to be found in the hypothesis here enunciated.



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## POSTMORTEM FEMORAL BONE MARROW STUDIES OF KALA-AZAR.

BY

HEMENDRA NATH CHATTERJEE,\*

*Department of Pathology Carmichael Medical College Calcutta*

Kala azar in the acute stage is difficult to diagnose either clinically or by laboratory methods. Its course is somewhat like that of the enteric fevers with a continuous pyrexia. In fact in not a few cases the disease is observed to be a prolongation of what was clinically an enteric infection. The spleen may be enlarged but not to the degree found in the chronic stage. The usual biochemical reactions of the serum such as the formaldehyde test or the urea-subamine test are either absent or indefinite and the characteristic morphological changes of the blood such as marked leucopenia, neutropenia and profound anaemia have not yet developed.

Even at postmortem examination there is a great liability to overlook the disease unless as a routine smears from spleen, bone marrow and liver are made. Kala azar is usually unsuspected as very few cases die in the acute stage. The usual causes of death at this stage are the various secondary affections. The following summary is from the records of such a case.

\* The writer is indebted for their help to Prof. C. C. BASU for facilities of work in the Department of Pathology, Carmichael Medical College, to Prof. M. N. DE and Prof. B. P. TRIBEDI for access to the pathological material of Medical College, Bengal, and to Dr. J. K. SARKAR for his whole hearted and active help.

The present investigation was carried out with the help of a grant from the Indian Research Fund Association.

A man aged 35 was admitted to hospital with fever for the past 12 days and great frequency of stools containing blood and mucus which gave an appearance of a reddish-brown green coloured liquid. Examination of stools showed large numbers of vegetative forms of *Entamoeba histolytica*. Blood 2 days after admission gave R.B.C., 3,600 000 W.B.C. 12,875 polymorph nuclears, 72 per cent. small lymphocytes, 26 per cent. large nil eosinophils, 2 per cent. The clinical diagnosis was acute dysentery with malaria and influenza.

*Postmortem Findings*—Large intestine showed amoebic ulceration. Haemorrhagic areas occurred in the left lung which sank in water. The spleen weighed 700 grammes and the liver 1 370 grammes. The naked-eye appearance of the bone marrow was that of formative marrow. Kala-azar in this particular case was not suspected until the microscopic examination of sections of bone marrow at a much later date showed the presence of a large number of clasmatocytes packed with leishmania. Sections from the spleen also showed the same parasites.

From the point of view of the changes which are found in the bone marrow in kala azar these may be considered as taking place in three stages.

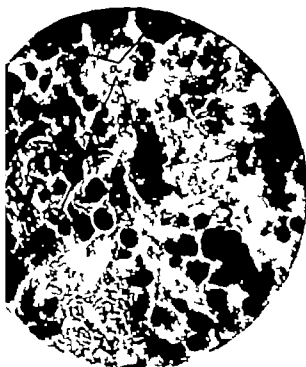
#### THE FIRST STAGE.

The marrow sections (Fig. 1) from the case described above showed almost complete absorption and replacement of the fat cells. The general structure consisted of a large number of clasmatocytes and also cells of myeloblastic series.

A differential count gave the following proportion of cells myeloid, 23 per cent. erythroid, 25 per cent. clasmatocytes 45 per cent. other cells, 7 per cent. Of the clasmatocytes about 30 per cent. were seen to be parasitised with leishmania. Some had ingested red blood corpuscles.

It was remarkable that there was a total absence of any of the degenerative changes such as are found in the subacute and chronic stages to be presently described. The absence of degenerative changes is all the more remarkable as there was no history of antimony injections. During life there had been very little clinical evidence of kala azar the diagnosis having escaped both the clinician and the morbid anatomist at the postmortem table.

The bone marrow of acute experimental kala-azar has been studied in laboratory animals. SHORTT (1923) studied the bone marrow of two *Macaca rhesus* which had received intraperitoneal injections of a mixed emulsion of liver and spleen from a human kala-azar case. The animals were killed on the 22nd and 34th days respectively. The bone marrow in the shafts of the long bones of the leg was dark red in colour and therefore in a hyperplastic stage. Microscopically the endothelial cells were greatly hypertrophied and crowded with parasites.



—Bone marrow from an acute case of kala-azar centre there is a clasmatocyte bowing leishmania. but the fat cells are almost completely absorbed.  $\times 950$  approx.—*Haematoxylin-eosin stain.*

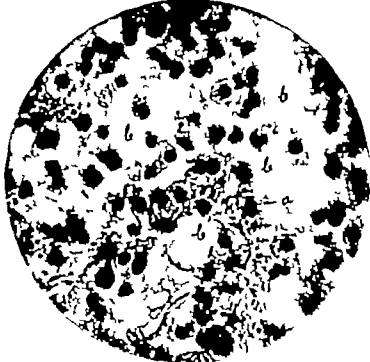
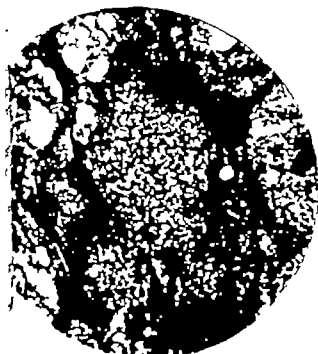


FIG. 2.—Bone marrow from a chronic case of kala-azar showing clasmatocytes (a) with leishmania. Fat cells (b) are numerous but irregular in outline.  $\times 950$  approx.—*Haematoxylin-eosin stain.*



—Bone marrow from the same case as Fig. 2, show gelatinous degeneration and precipitation of fibrin. the ragged appearance of fat cells.  $\times 650$ .—*Hesterblau stain.*

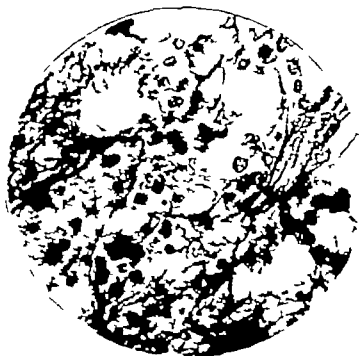


FIG. 4.—Bone marrow from the same case as Fig. 2, showing proliferation of reticular fibres.  $\times 650$ .—*Foot D elichowick stain*

our cases was as follows myeloid, 30 per cent erythroid 40 per cent. clasmatoocytes 5 per cent. other cells, 25 per cent.

In contrast to the first stage almost all the clasmatoocytes were parasitised.

These cells (see Plate) are much larger than myelocytes and possess protruding and extending protoplasmic processes which in the ordinary section are found to fade away into the general mass of ground substance. Consequently the shape and size of these cells vary greatly. The cytoplasm takes the eosin stain deeply. Detached portions containing only the parasites may be observed. The nucleus is usually elongated, rarely round and is eccentrically placed. Its shape is oval planoconvex or kidney-shaped. It is usually single but sometimes two or even three can be found in a single cell. It stains deeply with basic stains but not so intensely as the nuclei of the erythroblastic series of cells. On the inner surface of the nuclear membrane are found a number of chromatin granules. The nucleoli are often arranged in pairs, occasionally in threes and are placed eccentrically. A fine chromatin network is found to connect the chromatin granules and the nucleoli.

The general ground substance of the marrow consists of a somewhat glassy structure which to the naked eye exhibits what is called "gelatinous degeneration." It is unfortunate that the above term fails to give an idea of the change with which it is associated. As seen under the microscope in this condition the normally present fat vacuoles show a ragged and irregular periphery due to the precipitation of large amounts of a fine fibrillar substance consisting of fibrils which stain well with the specific stains for fibrin (Weigert fibrin stain) and also by Wasser blau and Unna's alkaline methylene blue.

These changes consisting of a fat disintegration as well as a process of precipitation of fibrin are not only present in kala azar marrow but also in that of various wasting conditions. Thus MICHAEL (1930) found it in order of frequency in tuberculosis and carcinoma and to a less extent in pyaemia and degenerative productive nephritis lymphogranulomatosis and ulcerative colitis. He also found it to be associated with comparatively advanced age and prolonged illness. DICKSON (1908) in earlier work threw a good deal of light on this condition. He pointed out the association of this condition with degeneration of fat. ASKANZY (1927) demonstrated the fibrin wreaths round the periphery of the fat vacuoles and in agreement with DICKSON considered that the flocculent precipitation is caused by cellular reactions.

There is increase of argyrophil reticular fibres in the marrow of kala-azar cases, but this change is more marked in the next stage.

### THE THIRD STAGE.

This is a more advanced stage. It has been possible to obtain the femoral marrow of three cases of this group. The following further changes can be observed microscopically

(a) The total number of cells have become very few the cells of the myeloid series being remarkably diminished. The general picture as seen with ordinary haematoxylin-eosin stain present a somewhat homogeneous ground substance with comparatively fewer and more dispersed cells, many of which are clasmato-cytes. The reticulo-endothelial cells or clasmatocytes are the most conspicuous cells especially those with a large amount of amoeboid and branched protoplasm. The majority of these cells are packed with the parasites (Fig 2.)

Along with these we also find a fair number of erythropoietic cells some of which are megaloblasts.

A differential count from the marrow of one of the cases was as follows myeloid cells 11 per cent, erythroid, 50 per cent, clasmatocytes 10 per cent, other cells 29 per cent.

(b) The fibrin deposition is more pronounced, the greater part of the ground substance consisting of a delicate mesh of the fibrin network which is well stained by the methods already mentioned. (Fig 3)

(c) In addition to the above changes there occurs marked proliferation of the reticular fibrils as demonstrated by Foot's Bielchowski stain. (Fig 4) These reticular fibrils differ markedly from the fibrin network in the following respects—

(1) They are argyrophil and take the stain well with the Foot's Bielchowski stain which does not stain the fibrin

(2) They are wavy and curly and discrete whereas the fibrin threads are straighter and have nodal thickenings, and intersections (FOOT 1925)

(3) They are not increased in other wasting conditions. This fibrinous degeneration of the bone marrow and the hyperplasia of the reticular fibres—two rather opposing features—form the characteristic finding in bone marrow of chronic kala azar

#### DISCUSSION

From the changes so far described, especially those in the second and third stages, it is not difficult to visualize the blood picture in kala azar

The great diminution of the cellular elements in the marrow and especially those of the myelocytic series in the advanced disease may account for the leucopenia and neutropenia. The appearance of the megaloblasts and a large number of erythroblasts may be associated with the increasing anaemia which occurs in this condition and which might ultimately turn into an anaemia of the macrocytic type (CHATTERJEE, 1939)

#### SUMMARY

- 1 The bone marrow changes in a case of acute kala azar are reviewed.
- 2 Various proliferative and degenerative changes in the sub-acute and chronic stages are described and their bearing on the morphological changes found in the peripheral blood is suggested.

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## A FURTHER BIOCHEMICAL STUDY OF THE BLOOD OF CHOLERA PATIENTS

BY

HEMENDRA NATH CHATTERJEE,\*<sup>1</sup>

*Department of Pathology Carmichael Medical College, Calcutta*

The present study is a continuation of a previous paper published in 1941 (CHATTERJEE and SARKAR, 1941a) and consists of the observations on the phenol content and the reserve alkalinity of blood of 55 cases of cholera. Observations on the non protein nitrogen have also been made in these cases.

The cases recorded constitute clinical cases of cholera as evidenced by vomiting, purging with rice water stools, anuria, muscular cramps, imperceptible pulse and signs of dehydration.

### THE PHENOL CONTENT

The figures obtained by the writer show a definite rise in the values of all the varieties of phenol (free, total and combined) of blood. The normal averages of the phenol content as have been found in a different study (CHATTERJEE and SARKAR, 1941b) of 20 normal persons are 2.24 mg. per cent. per 100 c.c. of blood.

\* The writer is taking the opportunity to offer his thanks to Dr B. C. ROY for facilities of work in the Cholera Ward, to Prof. C. C. BASU for similar facilities in the Pathology Department, to Dr J. SARKAR, Dr S. SEN and Dr S. M. GHOSH for their very valuable and sincere help.

<sup>1</sup> This work has been carried out under the auspices of Capt. KALYAN KUMAR MUKHERJEE, M.C., Research Scholar of the Calcutta University.



for free phenol, 0.39 mg. per cent. for conjugated phenols and 2.63 mg. per cent. for total phenols respectively. In contrast the average figures for these have been found to be 4.43 mg. per cent. 1.38 mg. per cent. and 5.80 mg. per cent. respectively in six cases of cholera whose blood was examined before any administration of saline transfusion. The highest and lowest and average values of these cholera cases will be found in Table I.

TABLE I  
FIGURES OF PHENOLS IN CHOLERA CASES BEFORE TRANSFUSION.

	Cholera Cases.			Average Normal Cases, mg. per cent.
	Highest, mg. per cent.	Lowest, mg. per cent.	Average, mg. per cent.	
Free phenol	5.0	3.3	4.23	2.4
Combined phenol	.68	0.02	1.38	0.39
Total phenol	6.3	4.17	5.61	2.63

As is well known a high specific gravity of blood is the usual finding in cholera. The following table (Table II) shows a few usual cases of cholera in which both the specific gravity of blood and its phenol content were high.

TABLE II  
CASES OF CHOLERA SHOWING THE SPECIFIC GRAVITY OF BLOOD AND ITS PHENOL CONTENT

Case No.	Specific Gravity	Free Phenol, mg. per cent.	Combined Phenol, mg. per cent.	Total Phenol, mg. per cent.
38	1.070	5.00	1.5	6.5
42	1.046	4.80	1.00	5.80
44	1.077	3.77	6.73	4.50
53	1.065	4.17	0.83	5.00

After following up the cases which received saline transfusions a somewhat increased phenol content has been observed to persist in a good number of them although the specific gravity was seen to come within normal limits. Thus in our series the increased phenol was observed to persist in 70 per cent. of our readings whereas high specific gravity was noticed only in 51 per cent. of the readings of our cases after saline transfusions.

In other words with transfusion of saline the specific gravity diminished and the phenol content tended to come close to normal but the figures of the latter were still somewhat higher than the normal average as will be evidenced by Table III showing two typical cases.

TABLE III

TWO CASES OF CHOLERA AFTER TRANSFUSION SHOWING SPECIFIC GRAVITY AND PHENOL VALUES.

Case No.	Date of Examination.	Saline Treatment.	Specific Gravity	Free Phenol, mg. per cent.	Combined Phenol mg per cent	Total Phenol, mg per cent.
28	6.6.40	Before treatment	1.070	5.00	1.25	6.25
R	7.6.40	After " "	1.060	2.80	0.30	3.10
"R	9.6.40	" " "	1.058	2.77	0.23	3.00
69	18.7.40	Before " "	1.056	3.57	2.68	6.25
"R	19.7.40	After " "	1.054	2.90	1.40	4.30

The increase in phenol is not due to concentration of blood as the same has been observed in three cases with low specific gravity of blood and great prostration, the so-called *vaso motor* type of cases.

TABLE IV

CASES OF CHOLERA WITH LOW SPECIFIC GRAVITY AND HIGH PHENOL CONTENT OF BLOOD

Case No	Specific Gravity	Free Phenol, mg per cent.	Combined Phenol, mg per cent.	Total Phenol, mg per cent.
26	1.054	3.10	1.00	4.10
29	1.058	4.17	0.83	5.00
70	1.056	4.12	0.88	5.00

On the other hand in four cases a low phenol content with a high specific gravity was observed. These were cases which came to the hospital early and were convalescent in a day or two. This is shown in Table V.

TABLE V

CASES WITH HIGH SPECIFIC GRAVITY AND LOW PHENOL CONTENT OF BLOOD

Case No	Specific Gravity	Free Phenol, mg per cent.	Combined Phenol, mg per cent.	Total Phenol, mg per cent.
70	1.063	1.62	0.42	2.08
24	1.064	2.50	1.00	3.50
7	1.068	2.38	0.84	3.12
30	1.064	2.50	0.30	2.80

## THE RESERVE ALKALINITY

The reserve alkalinity of whole blood (oxalated) was estimated by the method of LEVY ROWNTREE and MARRIOTT (1915). The same has been found to be on the acid side in the cholera cases. The average figures of oxalated whole blood of R<sub>p</sub>H in normal persons was found to be 8.2 to 8.5 in our institution (Dr H. N. MUKERJEE). There does not seem to be any direct association between R<sub>p</sub>H and phenol, although a low R<sub>p</sub>H and high phenol content might be present together. But the return to normal of the reserve pH seems to occur subsequently to that of specific gravity and phenol content, the specific gravity again becoming normal earlier than the phenol. (See Table VI)

TABLE VI.

PHENOL CONTENT, SPECIFIC GRAVITY AND RESERVE ALKALINITY OF BLOOD.

Case No.	Date	Specific Gravity	Free Phenol, mg. per cent.	Combined Phenol, mg. per cent.	Total Phenol, mg. per cent.	R <sub>p</sub> H, mg. per cent.
44	10.6.40	1.070	3.00	0.73	4.50	7.4
R	11.6.40	1.060	3.85	0.61	4.18	7.9
R	12.6.40	1.065	2.56	0.99	3.55	8.4
54	28.6.40	1.06	2.77	2.60	4.17	7.4
R	1.7.40	1.058	2.50	.50	3.00	7.4

## OTHER FEATURES.

The non protein nitrogen (N.P.N.) as is well known is generally raised above normal in cholera. The average value of N.P.N. in this series, before administration of saline was found to be 57.8 mg. per 100 c.c. of blood. As may be expected the transfusion of saline and glucose lowers the value of N.P.N. along with this the figures for R<sub>p</sub>H, specific gravity and phenol improve. Table VII shows the improvement in blood chemistry, running *pari passu* with improvement in clinical conditions of the patient.

## METHODS

Procuring blood from a cholera patient is a comparatively difficult procedure owing to its high concentration and great liability to clot within the syringe. The greatly collapsed condition of the veins is another difficulty. The following is a summary of the procedure adopted. Two workers are necessary for obtaining the samples and two 10 c.c. glass syringes with needles of identical size are used. One of the workers punctures a median cubital vein withdraws 10 c.c. of blood and removes the syringe while leaving the

TABLE VII

Case No	Date.	Symptom.	Specific Gravity	Free Phenol, mg per cent.	Combined Phenol, mg per cent.	Total Phenol, mg per cent.	RpH	N.P.N mg per cent.	Treat ment.
33	6.6.40	Vomiting purging anuria	1.070	5.00	1.25	6.25	7.6	56.0	Trans fusion
R	6.6.40	Purging	1.060	2.80	0.30	3.10	8.0	42.0	"
R	9.6.40	Convales cent	1.058	2.77	0.23	3.00	8.2	35.0	"
44	10.6.40	Vomiting purging anuria	1.0.0	3.77	0.73	4.50	7.6	63.0	Trans fusion
R	11.6.40	Improves	1.060	3.55	0.61	4.16	7.8	—	"
R	1..6.40	"	1.055	2.56	0.99	3.55	8.0	42.0	"

needle *in situ* From the syringe a drop is allowed to fall into each of the specific gravity bottles containing the various glycerine water mixtures for observation of specific gravity. The rest of the blood is transferred to a dry oxalated test tube, for the purpose of RpH determination of the whole blood.

The other worker has in the meantime, with the second syringe, withdrawn a further 10 c.c. of blood through the same needle and transferred it to another oxalated tube for the purpose of determination of the phenols.

RpH was determined by the method of LEVY ROWNTREE and MARRIOTT (1915)

The phenols were estimated by the method of THEIS and BENEDICT (1924) For the N.P.N. the micro-Kjeldahl method has been used

#### SUMMARY

1. A markedly high phenol content of blood was observed in all cases of cholera before administration of saline and figures somewhat higher than normal in 70 per cent. of readings of cholera cases after the administration of saline transfusion

2. The specific gravity of blood falls to normal with the transfusions but the increased phenol persisted in the above cases

3. Early and comparatively mild cases of cholera receiving prompt treatment showed a rapid return to normal of specific gravity as well as a normal phenol of blood.

4 There was a diminished reserve alkalinity of the blood. This persisted even after the specific gravity and phenol content of blood were brought down to normal.

5 The clinical improvement runs parallel with the improvement of the blood chemistry

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# A FIELD EXPERIMENT ON THE PROPHYLACTIC VALUE OF PENTAMIDINE IN SLEEPING SICKNESS

BY

L. VAN HOOFF R. LEWILLOX C. HENRARD E. PEEL

AND

B. RODJESTVENSKY

*Institut de Médecine Tropicale Princesse Astrid Léopoldville Belgian Congo*

An account of both curative and prophylactic action of pentamidine has been given in these TRANSACTIONS (VAN HOOFF *et al.* 1944). It was then stated that in 1943 a large scale trial under natural conditions of exposure to infection had been started on the prophylactic value of pentamidine against Gambian trypanosomiasis. This field experiment now affords sufficient data from which to draw some practical conclusions.

## I—THE EXPERIMENT

In a heavily infected focus of the Kwango District of Belgian Congo two villages were selected Iwungu and Mukwamene in which 1 124 natives lived. In 1942, ninety-nine new cases were found these, together with seventy old cases already under treatment and also the non permanent residents were excluded.

From those remaining in December 1942, 721 natives were given one injection of pentamidine on a basis of 0.003 gramme per kg body weight. The 213 left received no injection, and were selected as controls, so that they could share as equally as possible the chances of infection to which those under investigation were exposed, for example one woman or one child in each large family.

The fly density (*Glossina palpalis*) was high, though limited to characteristic breeding grounds near ferries at watering places and soaking places for manioc, along the palmo-forested banks of rivers and native plantations in half flooded fields. The infection rate of flies was not tested.

*First survey*, March-April, 1943.

694 injected negatives

New cases nil.

200 controls

New cases 6 (i.e. 3 per cent.).

*Second survey*, July 1943

679 injected natives

New cases nil.

194 controls

New cases 1 (i.e., 0.5 per cent.).

## II—FOLLOWING UP THE EXPERIMENT ON NEW LEVEL.

The 679 natives injected in December 1942, and still healthy in July 1943, were then divided in two sub-groups: 370 were injected again in July 1943, and 309 kept as controls. Isoethionic acid pentamidine salt was used in the dose of 0.004 grammes per kg. body weight as indicated by the drug manufacturer instead of pentamidine hydrochloride.

*Third survey*, November 1943.

361 natives injected twice

December 1942, and July 1943.

New cases nil.

332 natives injected once

December 1942.

New cases, 4 (i.e., 1.2 per cent.).

184 controls since December 1942

1 new case (i.e., 0.5 per cent.).

*Fourth survey*, February 1944

354 natives injected twice

December 1942, and July 1943.

1 new case (i.e., 0.3 per cent.).

331 natives injected once

December 1942. New cases, 4

(i.e. 1.2 per cent.).

193 controls

1 new case (i.e., 0.5 per cent.).

## III—CLINICAL ASPECT OF NEW PATIENTS DISCOVERED DURING THIS INVESTIGATION

At the start of this experiment, all the natives had been thoroughly examined. Those with enlarged cervical glands were punctured more than once. Lumbar puncture was performed on some of them, and thick blood films made of the whole population. During the survey the same procedure was applied, including the examination of the blood of all the natives. Although all these precautions were taken in Bayer 205 prevention in the bush, it was not unusual to find cases of sleeping sickness many months after injection of this particular drug. Such cases were, however, only discovered by the examination of cerebrospinal fluid. It has been suggested that Bayer 205 injected as a prophylactic has sometimes concealed the disease: the nervous

system was involved without any appearance of trypanosomes in the peripheral blood and the lymph glands. Fearing a similar process with pentamidine we insisted that all the new infections occurring during the investigation should be subject to lumbar puncture. But all of them (a total of eighteen) showed normal C S fluid for cell count as well as for albumin, globulin and absence of flagellates. No cryptic trypanosomiasis was favoured by the new prophylactic and this may be an important advantage.

#### SUMMARY AND CONCLUSION

Drawing attention to the fact that this investigation was conducted on the most practical lines using intentionally a single injection of a perfectly tolerated dose and involving the minimum of staff and technical work, it appears that the results are better than those obtained by other workers, as well as ourselves, with suramin drugs.

There are reasons to believe that in the field a single dose of 0.003 gramme per kg body weight injected in the muscles protects the healthy from sleeping sickness infection for at least 6 months with isethionic salt of pentamidine the suitable dose will be 0.004 gramme per kg body weight.

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# AINHUM A CLINICAL SUMMARY OF FORTY-FIVE CASES ON THE ISTHMUS OF PANAMA.

BY

B H KEAN

*Capt., Medical Corps Army of the United States*

HAROLD A TUCKER M.D

AND

WILLIAM C MILLER M.D

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## INTRODUCTION

Ainhum is a disease of negroes in which a band of constriction forms at the level of the digitoplantar fold generally of the little toe this constriction becomes progressively deeper until only a thin pedicle attaches the toe to the foot. The toe becomes bulbous and occasionally swollen. Eventually spontaneous amputation occurs. Excellent discussions and illustrations of the condition may be found in textbooks of tropical medicine (MANSION BAHR, 1943 STRONG 1942) SPINZIG's article (1939) is especially recommended.

Several hundred cases have been reported in the world literature since DA SILVA LIMA named the entity in 1867 but the majority of the articles describe only one or a few cases. Two reports have previously appeared from the Isthmus of Panama WEINSTEIN (1912) and MAXEL (1930) each reported two cases in West Indians living on the Atlantic side of the Isthmus.

During the 40-year period from May 1904 to May, 1944 there were 546 760 admissions to Gorgas Hospital. From the files of these admissions we were able to collect forty-two cases of ainhum. Three additional cases were obtained from the records of the Colon Hospital Surgical Clinic. The data on these forty five patients provided the basis of this report.

## THE DATA.

### *Incidence*

The incidence of ainhum at Gorgas Hospital was 1.5 per 10 000 male West Indian admissions. West Indian males represented approximately 50 per cent. of all hospital cases. The incidence per patient could not be obtained

because the 548,760 admissions to Gorgas Hospital included readmissions of the same individuals. No significant annual variation was noted the number of cases per year ranged from none to four

### *Race*

All of the patients were West Indian negroes most of whom had been "imported" to the Isthmus during the construction period of the Panama Canal, 1905 to 1913. Twenty of these patients were born in Jamaica, ten in Barbados, three in Antigua, two in Guadeloupe, and one each in Saint Vincent, Santa Lucia, Martinique, Fortune Island, Grenada, and Montserrat. In four, the birthplace was merely designated as "West Indies." No cases occurred among white or mestizo patients who comprised one third of the hospital population. The mestizos were Panamanians of mixed Indian and white descent.

### *Sex*

All of the patients were males. Although the ratio of West Indian males to females on the Isthmus has been approximately three to one, none of the cases of antrum were in females.

### *Occupation*

All were labourers except three seamen, two farmers, two waiters, a tailor and a laboratory worker.

### *Age of Onset*

The average stated age of onset was 35.9 years, the distribution according to decades being as follows —

11 to 20 years	3	61 to 71 years	—	1
21 " 30 "	13	Data not available	—	7
31 " 40 "	8			—
41 " 50 "	11	Total		45
51 " 60 "	2			—

In six patients evidence of the disease was present prior to arrival on the Isthmus. In thirty-one an average of 11.3 years elapsed after arrival before the onset of the condition. In eight, data on this item were not available.

### *Site of Involvement*

The little toes were involved in all instances. The right side was involved eighteen times, the left thirteen, and the fourteen remaining patients had bilateral antrum. Both little toes were often affected simultaneously but in one patient 18 years intervened between amputations. Involvement of toes other than the fifth occurred in one patient — the third, fourth, and fifth toes bilaterally were affected, but only the little toes required amputation.

### Family History

A history of familial anihum could be obtained but once the father of the patient had had the affliction of the corresponding toe. In five instances familial tendency was specifically denied and in the remainder no pertinent statement was made. WEINSTEIN (1912) reported a West Indian family living in the Canal Zone in which the patient, his father, brother and nephew were all affected.

### Symptoms

Pain was the presenting symptom in forty-four of the forty-five patients. This pain appeared late in the course of the disease and was of sufficient intensity to compel hospitalization for relief. Early pain was mentioned in twenty-one cases, in four of these a history of local trauma was obtained and in six corns or calluses were noted on the affected toes. In a single case late anaesthesia was said to have been present. Ulceration usually of minor degree was present in nine of the patients. These data emphasize the fact that anihum is a painful disease in some stages at least, despite widespread opinion to the contrary.

### TREATMENT

Amputation was performed in all cases. Results were uniformly satisfactory: the pain was relieved and did not recur. The average age of operation was 40.9 years with an average period of 5.0 years having elapsed from the time of onset. The duration of the disease varied from 1 month (questionable) to 20 years. In four patients with bilateral involvement, both toes were taken off at one time; in five the average interval was 7.4 years between operations with a maximum of 18 years. The interval was not recorded in the five remaining patients of this group but one of them had some years before removed the toe first involved with a chisel. Age distribution at the time of amputation was —

21 to 30 years	11	61 to 70 years	1
31 40	12	Not stated	1
41 50	11		—
51 60	9	Total	45
			—

### DISCUSSION

Various theories have been offered as explanation of this bizarre condition but for none of these is there good evidence (see SPINZIG 1939 for summary).

Although leprosy has been suggested as an aetiological factor, no evidence of that disease was found in the patients in this series. In a review of 110 autopsies on lepers performed at the Board of Health Laboratory during the period 1904 to 1944 no instances of anihum were encountered. Dr EZRA HURWITZ, the Superintendent of the Palo Seco Leper Colony, Canal Zone, has not seen anihum among 225 patients admitted to the colony.

Syphilis has been mentioned in this regard. In nineteen patients the Wassermann test of the blood was negative; in eight positive; in two doubtful,

and in sixteen the test was not performed. The incidence of positive Wassermann test in these patients was not significantly higher than in the general population of this stratum of society.

In no patient was a history of self mutilation obtained.

A particular search was made for mention of keloid tendency for it was thought that this might give a clue as to the aetiology of the disease. In only two instances was a definite tendency to keloid formation recorded. Tuberculosis and general arteriosclerosis of significant degree each was present in a single patient. Scleroderma was not mentioned at all.

Injury, irritation, and infection have also been blamed. It may be emphasized that these natives or their ancestors have been transferred from Africa to the West Indies and from there to the Isthmus of Panama, three places in which ainhum occurs but in which environmental conditions differ radically. For example, in Africa the natives do not wear shoes, in the West Indies some do and some do not, whereas in this series of cases most of the patients were shoe wearers.

In general, then, we may conclude that ainhum probably is not caused directly by environmental factors or other disease but is associated with an hereditary racial tendency. Sickleaemia offers a useful analogy.

### SUMMARY

The clinical data of forty five cases of ainhum on the Isthmus of Panama during the 40-year period 1904 to 1944 are summarized. The disease occurred in adult, active, otherwise healthy West Indian negro males, the incidence being 1.5 per 10,000 West Indian male hospital admissions. Pain was the prominent symptom. Amputation provided satisfactory results. No evidence of basic relationship between ainhum and other diseases such as leprosy, syphilis, scleroderma, and arteriosclerosis was noted, nor did environmental conditions appear significant. An hereditary racial tendency was believed to be the most important factor in the development of ainhum.

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## TICK-TYPHUS IN ABYSSINIA

BY

A. D. CHARTERS M.D. D.T.M. & H. CAPT. R.A.A.S.L.C.\*

Since no previous description of tick borne typhus in Abyssinia can be found in the literature, and seeing that the following cases differ in some respects from the disease which is endemic in Kenya and South Africa, an account of the series may be of interest. That the disease exists in Abyssinia has been claimed by the Army Pathology Laboratory Service in its *Current Notes* for December 1941. C. MANSON-BAHR (1942) has also recorded a case of tick-borne typhus in Addis Ababa, but stated that the patient must have contracted it in Somaliland.

The small outbreak occurred at Babile which lies at an altitude of 5 000 feet and is situated 17 miles from Harar. The patients were admitted to 8th (E.A.) Field Ambulance, to which unit the writer was Medical Officer. There were five cases one of which was a 'forme fruste' and will be described separately. One of the patients was treated by his Medical Officer Capt. R. M. CADENHEAD, during his bout of typhus but was admitted to 8th (E.A.) Field Ambulance for his orchitis.

\* I am indebted to Brigadier R. P. CORBIACK, O.B.E. Director of Medical Services G.H.Q. East Africa Command, for permitting me to publish this article. I wish to record my gratitude to Dr. GERTRUD THRIELER for her instructions on tick identification, which made possible a survey of the local species and to Capt. R. M. CADENHEAD for his co-operation and for sending me suspected tick vectors.

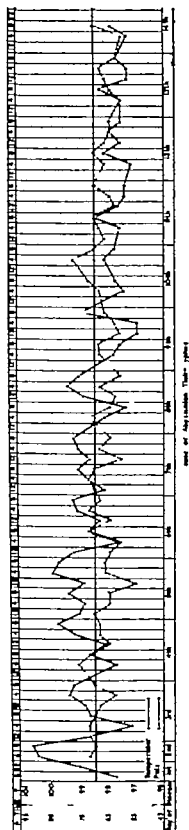
All cases occurred within the space of the three months, March to June, 1943 three of the patients, all from the same battalion, developing the disease during March. Only two units in the area were affected.

There was a history of tick bites in three cases, at periods of 5 days, 10 days and about 2 weeks respectively before the onset of symptoms. An oral lesion was present in two of the cases, the sites of the ulcers being the right groin and perineum respectively. Another patient, while exhibiting no primary sore suffered from a tender enlarged inguinal gland and gave a history of a tick bite on the umbilicus 2 weeks previously. The primary ulcer which was painless resembled exactly that which is associated with Kenya typhus. It was about  $\frac{1}{2}$  inch in diameter and had a black necrotic centre and indurated base. It was surrounded by a red areola. The lymphatic glands draining the affected area were enlarged and tender.

The onset of general symptoms coincided with the appearance of the primary eschar. A complaint common to all cases was a severe and intractable headache, out of proportion to the height of the temperature. Mental depression was also a prominent symptom. Joint pains (in left hip and left shoulder) were present in one case. The tongue was furred, but, apart from anorexia, there were no gastro-intestinal symptoms. The spleen could be felt in two cases, and the liver was both palpable and tender in one patient. One case exhibited a painless enlargement of his posterior cervical, axillary epitrochlear and inguinal glands.

The pyrexia (see Chart) was variable in character but was usually of irregular type. The maximum rises of temperature were  $102.4^{\circ}$   $102^{\circ}$  (two cases) and  $100.8^{\circ}$  F. The durations of fever were 7, 9, 12 and 14 days respectively.

The rash, which resembled that of Kenya typhus, appeared on the 4th day in one case and on the 6th day in two cases. (The fourth patient's history was uncertain.) The eruption consisted of slightly raised rose-coloured papules, which disappeared on pressure. As the result of the appearance of successive crops, the individual elements varied in size. The distribution, which was widespread, corresponded exactly to the Kenya form: no part of the body was spared even the face, palms and soles being affected. As in the Kenya disease, the rash tended to become more evident with rise of temperature and to fade when the fever dropped. In one case a thick crop of papules developed at the site of application of hot fomentations. Subjective symptoms referable to the eruption were almost completely absent. In one case only was there a complaint of slight itching in two papules, which differed from the remainder of the rash in being slightly more raised above the surface and in failing to disappear on pressure. In the writer's experience the spots of Kenya typhus never give rise to itching. The rash commenced to fade along with the subsidence of the pyrexia and, like its Kenya counterpart, left a faint stain which persisted for a few days after the fall of the temperature.





Owing to the absence of a haemocytometer total blood counts could not be performed, but the differential leucocyte counts are tabulated below —

	Case 1	Case 2	Case 3	Case 4
Time of count	4th Day	9th Day	During 1st Week.	During Orchitis.
Polymorphonuclears	35	36	81	88
Lymphocytes	40	60	39	30
Monocytes	23	4	0	4
Eosinophils	"	0	0	0

Weil Felix reactions were carried out by No. 1 Mobile Laboratory against *Proteus* OX19 OXK and OX2. All cases were negative with the exception of one patient, whose serum agglutinated *Proteus* OX2 in a dilution of 1:40 on the 10th day.

#### COMPLICATIONS

Unilateral (right sided) orchitis complicated the disease in two patients, commencing 11 and 14 days after the fall of the original pyrexia. The symptoms, which were similar in each case, comprised a tender enlargement of the testis and some rigidity in the right iliac fossa. There was a slight rise of temperature for 3 days. Examination of urethra, prostate and urine revealed no abnormality. There was no history of venereal disease. The durations of orchitis were 8 and 9 days respectively. Arising as these symptoms did, so long after the main febrile course, their rickettsial origin is not certain but the coincidence of two cases is suggestive.

#### FORME FRUSTE.

One patient, not included in the above series, developed mild symptoms suggestive of an abortive attack ("forme fruste") of tick typhus. He gave a history of having been bitten by ticks on his legs 7 to 10 days previously but did not remember a bite in the armpit. He noticed a sore in his right axilla on 12th June 1943. Two days later he complained of a headache and pain in his shoulder and at the same time observed that he had a rash.

On examination on 15th June he was found to have a typical *tick bite* sore in his right armpit and a tender axillary gland. The eruption consisted of characteristic rose-coloured papules and had the following distribution: twenty five papules on back, thirteen on chest and abdomen, seven on arms, three on face and one on right thigh. There was a painless enlargement of cervical, epitrochlear and inguinal glands: the tongue was clean: the spleen

and liver were impalpable the pulse rate was 90 per minute, but there was no rise of temperature. A differential leucocyte count performed on 15th June was as follows: polymorphonuclears, 36 per cent. lymphocytes 46 per cent. monocytes 14 per cent. and eosinophils 4 per cent.

The primary ulcer and rash began to fade on 17th June only 5 days from the date of onset of the disease, and had almost disappeared 2 days later. The headache and joint pains persisted for a total period of 3 days. At no time was the patient sufficiently ill to take to his bed or even to relinquish his normal (office) employment. A Weil-Felix reaction was not carried out.

Another patient received a second tick bite during his stage of convalescence. His temperature had been normal for 12 days and all previous signs and symptoms had disappeared. Although he suffered no ill health, his new sore refused to heal for 26 days. The ulcer which was situated in his left axilla and was accompanied by regional adenitis, resembled the primary eschar of tick-borne typhus. It appeared that his bout of typhus while promoting sufficient immunity to ward off a generalized infection failed to supply adequate protection against a primary lesion. Apparently the immune bodies of Abyssinian tick typhus like the agglutinins of its South African analogue do not reach maximum potency until convalescence is well established.

#### TRANSMITTING AGENT

An attempt was made to ascertain the arthropod vector of the disease. At the time of the epidemic the grass was long and ticks were numerous. One of the patients, a hygiene sergeant, performed duties which necessitated walking in the long grass. He suffered from a typical *tâche noire* in his right groin but did not remember being bitten. Another patient caught two ticks biting him 3 weeks before the onset of his attack, and gave them to Captain CADENHEAD. I identified these specimens as a male *Rhipicephalus pulchellus* and a female *Amblyomma marmoreum*. It is uncertain which of these species was the intermediary but as *A. marmoreum* was commonly found attached to reptiles (tortoises and puff adders) whose blood it is known to prefer it is probable that *R. pulchellus* was the vector.

A third patient gave a history of having been bitten by a small brown tick, which may have been *Haemaphysalis leachi* a frequent parasite of dogs in the district. A fourth patient drew a pepper tick from its attachment to his perineum. This was evidently the larval form, a stage in the life cycle which was extremely common during the rainy season and often caused distressing eruptions of the legs.

A collection was made of the prevalent Ixodidae in the area. They included *Rhipicephalus pulchellus* (cattle and dogs), *R. simus* (cattle and dogs), *Amblyomma variegatum* (cattle), *A. marmoreum* group (tortoise and puff adder) and *Haemaphysalis leachi* (dogs).

## COMMENTARY

There can be little doubt about the diagnosis of typhus. The history of a tick bite in three of the cases, the angry looking primary sore with its black necrotic centre and regional adenitis, the typical rash which appeared in crops and characteristically involved the palms, soles and face, the severe headache out of proportion to the height of the temperature and the course of the disease closely resembled the clinical features, not only of Kenya typhus, which has been excellently described by JEWELL and CORRIACK (1930) but also of the Tanganyika form (SHELLEY 1943) and the South African variety (GEAR, 1936; GEAR and BEVAN 1936). Moreover one of the patients suffered from rheumatic pains which are a common complaint in the Kenya disease.

The Weil-Felix reaction was admittedly negative in three out of four cases, but this test is not constantly positive in East African typhus. Moreover it is possible that one patient, whose blood was withdrawn on the 9th day would have given a positive result, had his serum been examined at a later date. PIJPER and DAU (1930) observed that in South Africa the Weil-Felix reaction only becomes positive during convalescence. GEAR states, "These agglutinins generally only appear after the 10th day of illness and reach the maximum titre well in convalescence so that tests taken during the fever usually give negative results."

The disease was less severe than Kenya typhus, and, like the milder form of tick bite fever in South Africa (GEAR) tended to have an intermittent temperature and shorter course. "Forme fruste" also occurred, as in tick-typhus elsewhere.

General adenitis, which is stated to be common amongst children and young adults in South African tick typhus (GEAR, 1941) was present in two patients, one of whom was 42 years of age.

Hepatitis was present in one case, this has not been observed by the writer in his Kenya cases.

In view of the fact that scrotal reactions follow the intraperitoneal inoculation of guinea-pigs with infected tissue, the complication of orchitis in two cases is of interest.

As in the South African variety there was a relative lymphocytosis in the early stages of the disease.

The limitation of the disease to Europeans, in spite of the predominance of Africans, was an interesting phenomenon.

A curious feature of the disease was its epidemic and local character. Three of the cases occurred in the same unit during a single month. A local incidence such as this has never been seen by the writer in Kenya,\* though GEAR and BEVAN (1936) working in South Africa, reported an instance of infection of husband and wife.

\* ROBERTS reported three cases of typhus in a Kenya brewery but produced evidence incriminating the rat flea, *Xenopsylla cheopis* as the infecting agent.

It has already been pointed out that the outbreak was confined to an interval of 3 months. Since Babile was employed as a military camping ground for 1 year only, it is reasonable to suspect that there may have been an epidemic season lasting from March till June. This hypothesis is supported by the fact that the rainy weather which prevails at this period, favours the reproduction of ticks.

That the vector was a tick is strongly suggested by the following observations —

- 1 There was a history of tick bites in three cases
2. While ticks were prevalent in the district, louse infestation was absent rats were not numerous and there were no histories of flea bites prior to the attacks. Although a mite of the genus *Trombicula* was common in the area the geographical limitation of mite borne typhus to the Far East, the absence in this series of *Proteus* O\K agglutinins and the more severe course of tsutsu gamushu testify against the incrimination of a member of the Trombididae as the transmitting agent.

- 3 The serum of one patient agglutinated *Proteus* O\2 in a dilution of 1/400 but gave negative results with *Proteus* O\19 and O\K. It is known from earlier work that cases showing agglutinins for *Proteus* O\2 only are almost certainly cases of tick bite fever.

Evidence has been adduced which suggests that *Rhipicephalus pulchellus* was a vector. ROBERTS and TONKING (1933) are of the opinion that the disease is caused by the same species in Kenya. It is interesting to note that *R. simus* which was found to convey the disease in Entrea (P. MANSON-BAHR, 1941) was also common in the Harar district. The Army Pathology Laboratory Service states that tick borne typhus in Abyssinia is conveyed by *R. sanguineus* a species which is also probably an agent in Kenya (ROBERTS and TONKING 1933). In South Africa the Ixodid vectors are stated to be larval forms of *Amblyomma hebraeum*, *Rhipicephalus appendiculatus*, *Boophilus decoloratus* (PIPPER) and *Haemaphysalis leachi* (GEAR and DOUTHWAITE, 1938). The prevalent ticks found at Babile have already been enumerated.

#### SUMMARY

- 1 A description has been given of five cases of tick borne typhus in Abyssinia.
2. The clinical features closely resembled those of East African and South African tick typhus.
- 3 The course of the disease was very mild one case of forme fruste being described.
- 4 Unusual clinical signs included general lymphadenitis and hepatitis.
- 5 Orchitis was a complication in two cases.
- 6 The blood showed a relative lymphocytosis in the early stages of the disease.

7 One patient, who was bitten by a tick during convalescence, developed a *tâche noire* without systemic symptoms.

8<sup>6</sup> The disease was confined to Europeans, in spite of the predominance of African troops.

9 A local and seasonal incidence has been noted.

10 A list of the prevalent ticks has been supplied.

11 *Rhipicephalus pulchellus* was probably a vector.

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## CORRESPONDENCE

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### HABITUATION OF *PULEX IRRITANS* TO ANIMAL HOST

To the Editor TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene

SIR,

BLANC and BALTAZARD'S successful transmission of plague to rats by the bites of *Pulex irritans* taken from plague patients in Morocco has once more aroused interest in the possible role of this species as an active vector of rat plague to man apart from direct conveyance from man to man in heavily septicaemic cases.

Although *P. irritans* has been found on many animals including rats I have encountered a belief perhaps based on some experiments of the Indian Plague Commission that such infestation is of a very fleeting character and that the flea of man does not adapt itself to an animal host. I should like to describe an experience of my own which proves the contrary. One warm summer a number of years ago our pet spaniel was found to be infested with *P. irritans*. The discovery was accidental, for the animal had never been seen to scratch and the invaders were difficult to detect in its black coat when brushed in the ordinary way. I kept the infested dog on my knees, carried it in my arms and came into direct contact with it in other ways on a number of occasions, yet found to my surprise that none of the insects deserted it for me, though ordinarily I am attractive to fleas. Finally the animal was fleeced with the help of a tub of warm water and cresol, an ethyl chloride spray, and a fine comb and over sixty fleas were recovered, all *P. irritans*. A few days later the operation was repeated when I counted over forty fleas of the same species. The fleecing had been carried out in an unoccupied room and knowing that a number of the

insects must have escaped capture. I entered the room several times subsequently wearing the minimum of clothing to see what would happen. Hunger proved to have overcome the inhibitions of the errant parasites, and I was viciously attacked about the ankles when I stood upright but when I tried the experiment of lying on the floor I was liable to be bitten on any part of the body—of some interest in considering the usual regional distribution of plague buboes. The dog's bedding was thoroughly dealt with, the house repeatedly "Hoovered," naphthalene and heating powder freely used, and so forth, yet in spite of these long continued measures, over a year elapsed before the dog was completely and finally rid of *P. irritans* though latterly it was present only in small numbers.

The infestation had lasted some considerable time before detection, for during a period of about 6 weeks preceding this I was aware of the occasional presence of a flea which when made captive proved to be *P. irritans*. My suggestion that the dog might be the responsible focus was scorned on the evidence given above. Possibly these were newly emerged adults which had not yet become addicted to dog's blood. At any rate except in the experiments mentioned already I was not assailed at any other time. Throughout the whole period the five other occupants of the house were never to their knowledge attacked. In other instances when in search of dog and cat fleas, I have proceeded to flea animals encountered casually and have found *P. irritans* in preponderating numbers (e.g. *Ctenocephalus* cases 2, *P. irritans* 30 + *C. felis* 3, *P. irritans* 9). There was however no way of discovering how long such infestations had lasted or the subsequent history of the animals in this respect.

In the past, English houses swarmed with fleas which Trevisa tells us spare not kings, and the Elizabethan Gascoigne speaks of symbolically as greedily gnawing his flesh and leaving the bones full bare. Judging from my experience given above some of the multitudinous *P. irritans* may well have habituated themselves to the house rat of the day and when these rodents died of plague have reinforced *Ceratophyllus* in an attack on man. The prevalence in old days of tokens (petechiae) and blains (blisters, white or black) suggests to my mind a larger proportion of virulent infections, possibly due to mass infection by hordes of vagrant fleas exceeding anything to be encountered to-day. These lessons have been so far forgotten in our time that Zinsser could actually write\* "There can be little doubt that the pestilence of Justinian [A.D. 542] was mainly one of bubonic plague, but the references to the general eruption of black blisters in many cases indicate that smallpox of a very severe type participated." Any Londoner of Shakespeare's or of Pepys's day would have recognized these "black blisters" as plague blains.

I am, etc.,

W. P. MAC ARTHUR.

## KALA-AZAR

To the Editor *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

I have read with great interest the paper on Recent Research in Kala azar in India, by Col H. C. SHORTT, which appeared in your September, 1945 issue\*. This is a most excellent presentation of the subject which many of us will find of great value.

I entirely agree with him regarding the great value of urea stibamine in kala azar. When however he quotes me as saying it is as effective as any of the newer preparations I think a little explanation is needed. Perhaps in my conversation I did not make myself quite clear.

In this part of India cases of kala azar occur in very large numbers and most patients have to be treated as out patients, which makes close medical supervision difficult. A drug for the treatment of kala azar in these circumstances needs to be cheap, safe and effective and easy to administer. This is where urea stibamine has proved so effective. It is relatively cheap and it is fairly safe, in the vast majority of cases of Indian kala azar it is highly effective. The only real difficulty is that it has to be given intravenously in young children, when repeated injections have to be given and the veins are sometimes blocked, this does sometimes create difficulty.

There are other preparations which may be slightly more effective in Indian kala azar neostibosan which was not cheap and has for several years not been available, and diamidino-stilbene which because of the toxic reactions and sequelae frequently produced, is unsuitable for the general treatment of kala azar in India. Our routine practice therefore, is to use pentavalent antimony, usually in the form of urea stibamine for the treatment of ordinary cases of kala azar and, in those few cases which do not respond, the patient is admitted to hospital and treated with diamidino-stilbene, which does appear to be effective in the few cases in which antimony is not.

I would point out that urea stibamine is a proprietary preparation, and that there are on the market apparently identical preparations which are equally safe and effective and even cheaper. One used in this institution in a large number of cases has been 'aminostiburea.'

There is no doubt, however, that for the treatment of kala azar a safe reliable and effective preparation that could be given intramuscularly would be a great advantage, particularly in the treatment of kala azar in young children which is very common. This was one advantage of neostibosan when it was available. Another preparation which could be given intramuscularly was solustibosan (sodium antimony gluconate) but a trial of this drug in the dosage recommended at that time by the manufacturers showed that the drug

\* SHORTT H E (1945) *Trans R Soc trop Med Hyg* 39, 13



was not very effective. An equivalent of solustibosan is "stibatun" (Gibbs). Similar preparations are being put on the market by other firms. In the discussion on Col. SHORTT's paper Dr EDMUND BURKE mentioned stibatun and the good results he had obtained. Recently other workers in India, including Dr P C SEN GUPTA in this institution, have been using stibatun with excellent immediate results but it is too early to express any opinion regarding the relapse rate—some relapses have already been seen. It is found, however, that the dosage originally recommended by the manufacturers of solustibosan and of stibatun and even the higher dosage used by BURKE, are not sufficient to control the disease in a considerable proportion of the cases.

The manufacturers of stibatun are now recommending higher doses. The dose recommended by P C SEN GUPTA is as follows (*Ind med Gaz.*, October 1945) for adults, a maximum of 200 to 240 c.c. according to body weight for older children the dose must be much higher in proportion to body weight, and from 150 to 200 c.c. is usually necessary for younger children 100 to 125 c.c. is usually enough. A similar high dose has been found advisable by K C CHAKRAVARTY (*Ind med Gaz.* October 1945). The high dosage in relation to the antimony content of stibatun is necessitated by the fact that antimony in this form is rapidly excreted, and the concentration in the body quickly falls below the therapeutic level. The same fact renders it desirable, if not necessary, to give injections every day. But time and careful study are needed before any final opinion can be expressed on the efficacy of this treatment of kala azar.

To sum up urea-stibamine and similar preparations are the most generally useful drugs we have in the treatment of Indian kala azar at the present time. Neostibosan or its equivalent, when it becomes available and if it is sufficiently cheap, may be preferable, mainly because it can be given intramuscularly. Sodium antimony gluconate may or may not prove equally effective, and can also be given intramuscularly.

Another point which might be mentioned regarding Col. SHORTT's paper is that we find sternum puncture of very great value in the diagnosis of early kala azar before the spleen has become sufficiently enlarged to be easily puncturable, and have obtained positive findings sometimes as early as 3 weeks from the onset of the fever—the percentage of positive findings in kala azar is something between 85 and 90 only slightly less than with spleen puncture in addition to being safer. The sternum puncture material is usually examined by direct smear and not by culture. Even in long-standing fevers, clinically kala-azar with marked splenic enlargement, we frequently do sternum puncture, and only if it is negative which is not often, is spleen puncture done.

Yours etc.,

JOHN LOVELL

Department of Tropical Medicine

School of Tropical Medicine, Calcutta.

## YELLOW FEVER IN WEST AFRICA 1942

To the Editor, *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

I was interested to see that ELLIOTT, in his paper entitled *Yellow Fever in the Recently Inoculated*, \* has published summaries of my autopsy reports on the two fatal European cases

In general, the pathological changes observed were characteristic of the disease, but we were particularly struck with the intense haemorrhagic lesions seen in the lungs. Such lesions have not always been recorded in West African cases of yellow fever (SMITH 1929) but KLOTZ and BELT (1930) state that they are found in as many as 90 per cent. of fatal cases, and BLAIR (1850) describes apoplexy of the lungs, with extravasation in some of his cases. The kidney lesions observed were similar to those described by many authors (KLOTZ and BELT 1930 SMITH, 1929 etc.) There was little change in the glomerular tufts. The cells of the convoluted tubules showed many stages of granular degeneration and the lumina of the tubules contained both granular epithelial debris and hyaline casts. In one patient the medullary vessels were congested and there were some haemorrhages into the tubules. The cortex was relatively anaemic. We have suggested elsewhere that these lesions may have arisen from renal anoxia (MAEGRAITH, HAVARD and PARSONS 1945)

One or two points in ELLIOTT's paper need correction. First, his statement that macerated liver tissue was injected into *Macacus rhesus* monkeys is incorrect. We used *Cercopithecus nigrifacies*. Secondly ELLIOTT entitles his paper *Yellow Fever in the Recently Inoculated*. I think it is only fair to point out that there is considerable doubt as to whether in fact, the deceased patients were inoculated with active yellow fever vaccine. FINDLAY and I went into this question carefully some time after the event and found that there had been a good deal of evasion of inoculation amongst the troops concerned before leaving England. One of the deceased, in fact, had been known to boast of his skill of evading the needle.

I think the real lesson we learned from this outbreak of yellow fever was the value of immediate immunisation of troops with active vaccine properly handled. Immediately after the diagnosis was established all troops in the Colony were reinoculated, starting with the Unit in which the outbreak had occurred. No further cases of yellow fever were reported. The task of reinoculation fell to me and involved taking tested vaccine on ice all over the Colony concerned. I have never decided which frightened me most flying in a "Walrus" over solid African jungle, or watching *Aedes* mosquitoes hopping about just over my head in the shambucks of the infected camp during the first days of the outbreak.

\* ELLIOTT MOUNTJOY (1944) *Trans. R. Soc. trop. Med. Hyg.*, 28, 231

The temperature and pulse charts ELLIOTT published might, I think, have received a little more of his attention. It is not clear from his table to which of Faget's signs he is referring. As the temperature and pulse rate charts of his Case 1 illustrate all Faget's signs so clearly it might be worth while quoting from that authority himself if only to point out that the so-called "Faget's sign" is, in fact, two signs: one diagnostic, the other prognostic. "*dans la fièvre jaune dès le début la ligne du pouls descend pendant que celle de la température se maintient horizontale* dans l'immense majorité des cas, ou même *monte* pendant deux, trois jours et davantage, dans les deux tiers des cas au moins. Voilà le signe clinique pathognomonique que de la fièvre jaune.

La discordance initiale des deux lignes du pouls et de la température dans la fièvre jaune voilà donc, son *signe clinique pathognomonique*.

Les deux lignes parallèlement descendantes du moins à partir du troisième ou quatrième jour lentement et persévéramment descendantes, voilà ce qu'il y a de plus rassurant quand on suit attentivement la marche de la fièvre jaune. Enfin plus tard encore, les deux lignes viennent-elles à montrer une *divergence inverse de celle du début* celle du pouls prend-elle une marche ascendante à la fin pendant que celle de la température se précipite en bas, la mort est à peu près certaine. On plutôt elle est même proche.

Yours sincerely

BRIAN MACGRAITH.

Department of Tropical Medicine,  
Liverpool School of Tropical Medicine.

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TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

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VOL. XXXIX. No 5 APRIL, 1946

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ORDINARY MEETING  
of the Society held at  
Manson House 28, Portland Place, London,  
on  
Thursday 17th January, 1946, at 8 p.m

THE PRESIDENT  
C. M. WENTON C.M.G. C.B.E. M.B. B.Sc., F.R.S.,  
in the Chair

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PRESENTATION

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THE THEOBALD SMITH GOLD MEDAL.

The President Ladies and Gentlemen, we are very much honoured this evening in having with us the United States Ambassador The Honourable JOHN GILBERT WINANT and before we proceed to the usual business of the meeting, His Excellency would like to make a statement.

The Honourable J G Winant, who was greeted with enthusiastic applause, said I am happy to come here this evening at the request of the officers of the American Academy of Tropical Medicine, to assist in doing honour to your distinguished President, Dr CHARLES MORLEY WENTON

Today we believe that the men of all nations must work together for the common good. Our governments and our peoples are giving deep thought and earnest effort to establishing channels of communication through which men may learn to understand other cultures and so find the common denominators of purpose. They are endeavouring to set up machinery through which economic, political and social co-operation may be established. We have faith in the ultimate success of these efforts though the way is difficult. We find new encouragement when we turn to the world of science where co-operation has long been a living fact and great men work selflessly in the pursuit of truth

for the benefit of mankind. During this war British and American scientists have worked together more closely than ever before—joint efforts have given to the world the benefits of penicillin, DDT and radar.

I wish to speak of Dr. WENTON not only as an eminent man of science, an authority on tropical disease, but also as a world citizen. During this global war soldier and layman have become increasingly aware of his contribution in life-saving. Even greater than the hazards of high explosives were the dangers of disease in desert and jungle. Yet the casualties from these causes have been lower than we dared to hope. We are beginning to understand something of what the advances in knowledge of tropical disease mean to millions of people living in infected areas. Dr. WENTON has played a great part in this through his own investigations of protozoan diseases and of malaria and by the leadership and guidance which he has given to others in this field.

Dr. WENTON on behalf of the American Academy of Tropical Medicine, I present to you the THEOBALD SMITH GOLD MEDAL. As a student, you so distinguished yourself at the University of Leeds, Guy's Hospital and the Pasteur Institute that you were called by that great pioneer in tropical medicine, Sir PATRICK MANSION to be the first protozoologist at the London School of Hygiene and Tropical Medicine. You later became Director of the Bureau of Scientific Research of the Wellcome Research Institution and then Director in Chief of that Institution. You now carry on as an honoured consultant and leader. Your laboratory has been in many lands—the Sudan, Malta, Mesopotamia and Greece. As one surveys your career one feels a strong element of the romance of exploration as well as of tireless labour. Your name and work are known not only to those who are privileged to call you friend, but also to countless numbers you have benefited.

I understand that you are already an Honorary Member of the American Academy of Tropical Medicine. You now become the first man outside America to receive the THEOBALD SMITH GOLD MEDAL. Your American colleagues take great satisfaction in this award. They only regret that it has not been possible for you to visit them personally to receive it.

I take great pleasure in reading the citation and in handing to you a medal which is a symbol of the esteem in which you are held by the scientists of the world.

#### *The Citation*

On behalf of the American Academy of Tropical Medicine this Theobald Smith Gold Medal is conferred on

CHARLES MORLEY WENTON

pioneer in tropical medicine. He has brought light when there was darkness, not hesitating to experiment on himself, he has obtained direct answers to many important questions in disease transmission, and through his discoveries he has made the world a better place to live in for all peoples without regard to race, creed, colour or economic status. He is one of those leaders who are to the everlasting credit of the British Empire."

The Ambassador then presented the Medal to Dr WENTON—with a copy of the citation.

Dr C M. Wenyon (in reply) Your Excellency, I must thank you for the very kind words you have spoken, and would ask you to convey to the PRESIDENT and COUNCIL of the AMERICAN ACADEMY OF TROPICAL MEDICINE my appreciation of the great honour they have done me in awarding me the THEOBALD SMITH GOLD MEDAL with which, on their behalf you have just now so graciously presented me.

I am all the more gratified by the award when I reflect that THEOBALD SMITH made one of the most important discoveries in protozoology—the only field of science in which perhaps I can lay any claim to distinction.

It was over half a century ago in 1889 in fact, that THEOBALD SMITH described a protozoan organism, now known as *Babesia bigemina* which he had discovered in the blood of cattle suffering from Texas fever or red water fever as it is more generally termed. This in those days was a remarkable enough observation but, not content to rest on his laurels, he set to work with KILBORNE on a long series of laborious investigations into the method of spread of the infection, then an unsolved problem which appeared to be connected in some way with the soil. These were crowned with success for 4 years after his announcement of the discovery of the organism he had proved not only that it was transmitted by the cattle tick *Boophilus annulatus* but that it passed from one generation of tick to the next through the egg. This is necessarily so for the tick is a one host tick which, having gained a footing on an animal in the larval stage, remains on the skin for the rest of its life, feeding on blood till the fertilized female finally falls off to lay her eggs on the ground before dying. In due course larvae hatching from the eggs crawl up blades of grass and quickly infest any cattle which come into contact with them. If the host from which the female tick dropped in the first place harboured the parasite in its blood, then the female tick itself was infected and passed the infection by way of its eggs to all the larval ticks which hatched from them. Such infected larvae never failed to infect any susceptible cattle from which they took their first feed of blood. All this and much more was described in detail by THEOBALD SMITH and KILBORNE in the first Bulletin issued in 1893 by the U S Department of Agriculture, a publication which is a classic and a model of what such a report should be. It is one which should be read by everyone seriously interested in the arthropod transmission of disease.

This indeed, was a stupendous discovery the first demonstration that a blood-sucking arthropod could transmit an infection by its bite. It was the forerunner of a series of similar discoveries made by workers in our two countries in which blood sucking arthropods were incriminated as vectors of a number of diseases—malaria and yellow fever by mosquitoes sleeping sickness by tsetse flies plague by fleas and various forms of typhus and relapsing fever by lice and ticks—to mention only a few.

When the American Academy of Tropical Medicine was founded in 1934

it was most appropriate that THEOBALD SMITH should be elected its first President, in recognition of his epoch-making discovery in Protozoology—a subject which is of such great importance in Tropical Medicine. The Council of the Academy then paid me the great compliment of electing me one of its first three Honorary Members.

THEOBALD SMITH died a few months after his election as President, and the Gold Medal which you, Sir, have this evening presented to me, was founded in his honour. This is the fifth award of the medal, the previous recipients being BARBER, STRONG, STITT and CRAIG—names which are too well known throughout the world in the field of Tropical Medicine to need any comments from me. They are all Americans and I may perhaps be excused if I take some pride in the fact that I am the first representative of another nation, albeit a very closely related one, to receive this high award. I accept the medal at your hands, Sir, in all humility, realizing that the honour is not for me alone but is a recognition by the American Academy of the important part Great Britain and its Empire have played in the development of tropical medicine.

During the war our two nations have been thrown very close together in many tropical centres of activity. In the field we have been jointly occupied in the solution of many tropical medical problems, while knowledge acquired by investigations carried on in our respective countries has been fully shared to our mutual advantage. Now that the war is over it is to be hoped that the close association and exchange of information will be continued and that the many new contacts and relationships which the war has brought about will not be broken. It appears to me that this award by the American Academy of Tropical Medicine sets a seal to the continuance of this mutual respect and understanding which have long existed between investigators in our two countries in the field of tropical medicine.

I must again thank you, Sir, not only for honouring our Society by coming here this evening, but also for giving me the very great satisfaction of receiving at your hands the Theobald Smith Gold Medal of the Academy of Tropical Medicine of the great country which you represent—a country which has made and is still making such valuable contributions to knowledge in this branch of medicine. Your country and mine have very great responsibilities for the health and well-being of the millions who are destined to pass their lives in tropical lands, where they are beset by many and often mysterious dangers, depend to a large extent on our knowledge of the diseases of these areas and, above all, on the successful application of this knowledge by the men and women we train to put it into practice. In this respect our purpose is one, which may very suitably be expressed by the motto of this Society—the Royal Society of Tropical Medicine and Hygiene—*ZONAE TORRIDAE TUTAMINI*.

The Ambassador then took leave of the President and Fellows and, after signing the Visitors Book, left Manson House.

## PAPER

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### MEDICAL DISORDERS IN EAST AFRICA.

BY

E. R. CULLINAN M.D., F.R.C.P.,

*Physician, St. Bartholomew's Hospital late Consulting Physician East Africa Command*

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#### I A SURVEY OF MEDICAL DISORDERS

#### II. THE NEED FOR CO-ORDINATED CLINICAL INVESTIGATION IN THE FUTURE.

The first part of this paper contains a brief survey of medical disorders which affected British troops in the East Africa Command during the last 20 months of the recent war. The second part makes a plea for the institution and encouragement in the immediate future of co-ordinated clinical investigation in the East African territories.

#### I A SURVEY OF MEDICAL DISORDERS

During the last 20 months of the war there were great opportunities in the East Africa Command for studying racial differences in disease. The Army was in training for service in South East Asia and thus was fairly static. It was composed mainly of native Africans and Europeans. It comprised a cross-section of the fittest part of the young adult male native population of the countries of East Africa, and of a representative sample of adult Europeans. These two groups of people, differing so widely in race and normal domestic habit, were living under very similar conditions in the same tropical countries. The East Africa Command is geographically large and the climate varied. It extends from 11° north to 25° south of the Equator and occupies over one million square miles. It includes the territories of Kenya, Tanganyika, Northern Rhodesia, Nyasaland, Somalia (Italian Somaliland) British Somaliland the reserved areas of Abyssinia Madagascar Mauritius, the Seychelles and Zanzibar (See map p 356)



Tables I and II show respectively the number per 1 000 of European and non-European troops suffering from medical disorders admitted to military hospitals in the Command for the 12 months from 1st July 1944, to 30th June, 1945. The non-European troops were mostly native Africans. In the Army all patients who are ill are admitted to hospital so that the figures give a reasonably accurate picture of the incidence of disease in the particular groups of people concerned.

TABLE I

EUROPEAN TROOPS—NUMBER PER 1 000 SUFFERING FROM MEDICAL DISORDERS ADMITTED TO MILITARY HOSPITALS IN THE EAST AFRICA COMMAND FROM 1ST JULY 1944, TO 30TH JUNE, 1945

Malaria	108.86	Other infectious diseases	...	3.47
Dysentery and enteritis (including amoebiasis)	66.79	Diseases of central nervous system	...	3.25
Upper respiratory infections	41.38	Diseases of blood	...	3.20
Diseases of skin (excluding tropical ulcers)	79.49	Tuberculosis (all forms)	...	1.83
Diseases of digestive system	76.28	Diseases of endocrine gland	...	1.41
Pyrexia of uncertain origin	19.26	Dengue	...	0.85
Psychoneurosis	17.59	Rheumatism—acute	...	0.71
Diseases of bones, joints, fasciae etc.	11.26	Relapsing fever	...	0.60
Typhus fever syndrome	9.84	Schistosomiasis	...	0.60
Bronchitis	9.42	Chickenpox	...	0.58
Other respiratory diseases	7.65	Measles	...	0.44
Infective hepatitis (including post-erysipelas jaundice)	7.76	Herpes zoster	...	0.44
Diseases of circulatory system	6.98	Enteric group fevers	...	0.32
Diseases of excretory system (excluding schistosomiasis)	6.01	Acute anterior poliomyelitis	...	0.28
Helminthiasis	5.57	Glandular fever	...	0.27
Pneumonia—acute (including atypical pneumonia)	4.83	Undulant fever	...	0.25
		Diseases of metabolism	...	0.17
		Mumps	...	0.17
		Vaccinia	...	0.14
		Deficiency diseases	...	0.06
		Other medical disorders	...	3.13
Total				401.61

Veneral disease (itself a serious problem in East Africa), tropical ulcers, diseases of the eye, ear, nose and throat, are not included in the tables.

It will be seen that the most prevalent diseases in both Europeans and non-Europeans were malaria, dysentery and enteritis, and upper respiratory infections, in that order. After those, however, the prevalence of other diseases differed markedly in the two groups. For instance, dengue and the typhus fever syndrome were mostly found among Europeans, schistosomiasis, relapsing fever, leprosy and yaws were almost exclusively found among non-Europeans.

Other diseases which were more common in Africans included meningitis, smallpox, chickenpox and mumps. It is particularly interesting that digestive disorders were nearly five times, skin diseases over three times, and psychoneurosis over twice as frequent in Europeans.

TABLE II

NON-EUROPEAN TROOPS—NUMBER PER 1 000 SUFFERING FROM MEDICAL DISORDERS ADMITTED TO MILITARY HOSPITALS IN THE EAST AFRICA COMMAND FROM 1ST JULY 1944 TO 30TH JUNE, 1945

Malaria	43.46	Diseases of excretory system (excluding schistosomiasis)	1.77
Dysentery and enteritis (including amoebiasis)	21.50	Diseases of central nervous system	1.61
Upper respiratory infections	16.51	Leprosy	1.47
Bronchitis	15.14	Measles	1.21
Relapsing fever	10.49	Meningitis—acute	0.90
Pneumonia—acute (including atypical pneumonia)	9.39	Yaws	0.71
Diseases of skin (excluding tropical ulcers)	8.72	Smallpox	0.36
Psychoneuroses	7.84	Enteric group fevers	0.35
Schistosomiasis	*7.64	Other infectious diseases	0.31
Other respiratory diseases	7.21	Diseases of endocrine glands	0.23
Diseases of circulatory system	6.78	Deficiency diseases	0.21
Pyrexia of uncertain origin	6.60	Herpes zoster	0.16
Diseases of digestive system	5.70	Rheumatism—acute	0.12
Infective hepatitis (including post-erythemaemic jaundice)	5.46	Glandular fever	0.11
Tuberculosis (all forms)	4.14	Undulant fever	0.09
Diseases of bones joints fasciae etc.	4.01	Encephalitis—acute	0.09
Helminthiasis	*3.23	Vaccinia	0.08
Chickenpox	2.93	Diseases of metabolism	0.06
Mumps	2.38	Kala-azar	0.03
Diseases of blood	1.86	Dengue	0.04
		Typhus fever syndrome	0.04
		Acute anterior poliomyelitis	0.03
		Other medical disorders	1.78
Total 205.74.			

\* Note.—Figures do not give a true picture of incidence

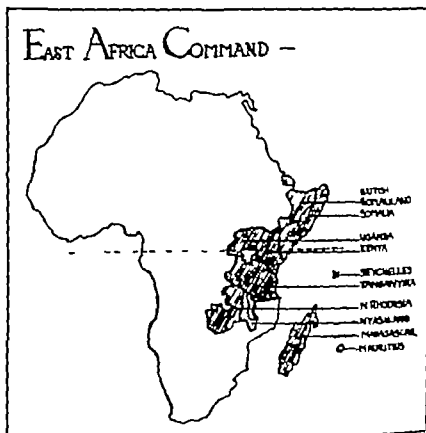
TABLE III

PERCENTAGE CASE MORTALITY FOR VARIOUS MEDICAL DISORDERS FROM 1ST APRIL, 1944 TO 31ST DECEMBER, 1944

	European.	Non-European.
Malaria	0.2	0.1
Dysentery (including amoebiasis)	0	0.6
Pneumonia—acute	0	3.1
Tuberculosis (all forms)	4.0	15.7
Other respiratory diseases	0.8	0.6
Relapsing fever	0	0.2
Jaundice	2.9	2.6
Meningitis—acute (2 deaths)		21.7
Diseases of central nervous system	11.4	4.0
Diseases of digestive system	0.2	4.3
Diseases of circulatory system	0	1.7
Diseases of blood	2.0	3.3
Diseases of excretory system (excluding schistosomiasis)	0	7.5
Other medical disorders	0.1	0.2

Table III shows the percentage case-mortality of the various medical disorders for the 9 months from 1st April, 1944 to 31st December 1944. The killing diseases of the African were tuberculosis, meningitis and pneumonia. In the year 1944 they accounted for 18.0 per cent. 12.1 per cent. and 11.7 per cent. of all non-European deaths.

It is impossible in this paper to make more than a few very brief remarks about some of these diseases.



#### MALARIA.

Nearly all malaria in the mainland parts of the Command is caused by infection with *Plasmodium falciparum*. In a few districts, such as parts of Somaliland, as well as in Madagascar and Mauritius infection is also caused by *P. vivax*. In Mauritius, and very rarely elsewhere, *P. malariae* infections occur.

The malaria season varies greatly from region to region, while in uncontrolled and highly hyperendemic areas transmission may be almost perennial. BAGSTER WILSON (1944) told me that people in East Africa who have lived all their lives in districts where malaria transmission continues for 6 months or more annually develop immunity to malaria. This immunity is labile, depending for its being on frequent occurrence of reinfection. He has mapped out the districts, and has also shown that all Europeans, Asians and certain African tribes, including the Somali, Kikuyu, Chagga and others coming from high altitudes, should be regarded as 'non immune.'

Whereas malaria in the non-immune may be of all grades of severity, in the immune it is almost universally mild, and the difference between the clinical pictures in the two groups may be so great that much confusion may arise in diagnosis unless this difference is constantly borne in mind.

The two common errors in diagnosis are believing a non immune to have another disease when he has malaria, and believing an immune to have malaria when he has another disease. A patient, especially an immune African, may equally die of some other disease, such as pneumonia or cerebrospinal meningitis, which would respond to specific therapy if a correct diagnosis were made in time. Nevertheless few mistakes were made when the investigation of a patient was careful, thorough and prompt. Malaria has been called the 'great mimic' maybe it is but a too eager acceptance of this comfortable phrase leads to slovenly thought in diagnosis. Indeed, the more carefully one studies the features of this 'great mimic' the less one is deceived by its miming.

Malaria among the non immune group usually ran a fairly typical and not too severe course. Nevertheless, from time to time most of the severe types of the disease were seen. Some patients suffering from cerebral symptoms developed these symptoms in hospital while being treated with quinine. Occasionally the blood of a very ill patient showed schizonts and amoeboid forms of *P. falciparum* which increased in number during treatment. Other patients sometimes showed the dangerous 'thickened' forms of parasites in their blood. Algid forms of the disease were rare. Blackwater fever was uncommon and responded favourably to early treatment. Jaundice indistinguishable from infective hepatitis appearing synchronously with malaria, but clearing rapidly with anti-malarial therapy caused much perplexity. So-called 'hepatic' malaria was not encountered. A few patients suffering from malaria had signs, apparently for the first time, of nephritis. Two of them died with symptoms of acute renal failure. The histological findings were most interesting: the kidneys showed, in addition to the tubular degeneration usually described, diffuse glomerular infiltration. Dysenteric symptoms were not infrequent concomitants.

RAPER, OGBORN and BAGSTER WILSON (1944) have shown that it is neither

necessary nor desirable to give an immune African a full course of treatment for malaria. Treatment of the non immune and semi immune groups followed standard practice, except that plasmoquine was not given as a routine. It was considered unnecessary to protect the local mosquitoes, who already had a large human malarial pool available, from the chance of acquiring the disease from a patient during the 12 days or so when he remained infective after treatment.

If properly treated, malaria in Europeans caused by *P. falciparum* in East Africa is nearly always completely curable and relapses, except occasionally immediately after a course of treatment are most uncommon. Such treatment is easy to carry out with Service personnel, but less easy with the general public. There is among the European population especially among those who have lived there many years, a tendency to look on a "touch of fever" with lamentable equanimity and disregard. Their pathetic faith in the efficacy of "5 grains of quinine" or more still, of "an injection, smacks more of ancient magic than of modern medicine

#### BACILLARY DYSENTERY

With few exceptions, the clinical course of bacillary dysentery was mild, and the percentage case mortality was low. As usual, the fatalities nearly all resulted from dehydration. Most of those who died were grossly dehydrated and emaciated on arrival at hospital, often dying shortly after admission in spite of prompt and adequate treatment. Africans particularly suffering from bacillary dysentery travel badly and dehydrate quickly. They should be treated as soon as possible after the onset of symptoms and as near as practicable to the place where the disease originates.

In the treatment of Africans, very large quantities of fluid were often required. Sulphapyridine seemed as effective as sulphaguanidine and for some unexplained reason does not upset the African as it does the European.

#### AMOEBIASIS

As the years of war went by and European troops were spending increasingly longer times in the tropics the incidence of amoebiasis rose among them. In 1944 the number of European troops admitted for amoebiasis corresponded to 20·27 per 1,000

Treatment, in general, was along conventional lines preliminary treatment with emetine hydrochloride by injection followed by a course of emetine bismuth iodide by mouth and retention enemata of quinoxyl, and then carbarsone or stovarsol. As in other theatres of war everyone was struck by the ease with which dysenteric symptoms could be controlled and by the difficulty of later

sterilizing the bowel of cysts or of being sure that a patient was cured at the end of a thorough course of treatment. Many of the European cases relapsed, not only those who persistently passed cysts in their stools. Many of them had recurrent attacks of the disease. Whether these recurrences resulted from reinfection in susceptible individuals or were caused by the development of bowel lesions, however minute, was impossible to say. These patients were so difficult to control that when they had frequent recurrences they were usually sent home to the United Kingdom.

#### SKIN DISEASES.

Skin diseases were relatively uncommon in African troops. Even in European troops skin diseases were frequent only in humid stations such as Mogadiscio in Somalia, the Seychelles and Diego Suarez. In the latter town prickly heat, furunculosis and otitis externa were common ailments. I have deliberately omitted tropical ulcers from this survey but I must remark that these lesions which were so prevalent in the African troops in the earlier part of the war, had towards the end of the war almost completely disappeared.

#### PSYCHONEUROSIS.

It is fascinating to compare the psychoneurotic disorders suffered by the civilized European and by the relatively uncivilized African. The majority of the European patients suffered from anxiety states, the majority of the African from hysteria. Thus JAMES (1944), in a series of psychoneurotic patients seen by him, reported anxiety states in 181 out of 333 Europeans (54.4 per cent.) and only 25 out of 364 Africans (6.4 per cent.) On the other hand, 206 of the 364 Africans (56.6 per cent.) suffered from hysteria.

European troops admitted to hospital for psychoneurosis in 12 months corresponded to a rate of 17.59 per 1000. This figure is not really high especially when it is realized that static conditions of war are as great a cause of mental breakdown as active conditions: prolonged absence from home, loneliness, and lack of action are powerful enemies to all but the perfectly adjusted mind.

#### RELAPSING FEVER.

Relapsing fever is common in many parts of East Africa. It is largely confined to the native population. The disease is tick-borne except in Abyssinia, where the louse-borne type is also found. The responsible tick vector is the *Ornithodoros moubata*. In Somaliland, *O. savignyi* has been suspect, although an outbreak which I saw there last year was almost certainly caused by the bite of *O. moubata*.

The incidence of 10.49 per 1 000 non-European troops shown in Table II is unusually high. It resulted from a large number of cases arising in a big details camp which had become heavily infested with *O. morbita*. Relapsing fever in adult East Africans is milder than in Europeans. Whatever else may explain this, it is probable that natives living in an endemic area develop some degree of immunity. Thus they believe themselves they have an objectionable habit of carrying round in a match box a tick, which is thought to be infected, which they let out to feed on themselves from time to time.

The incubation period appeared to range from 4 to 18 days. Spirochaetes were commonly not seen in the first blood slide examined, but were almost invariably found in subsequent ones.

It was noticed that a relapse was frequently "missed" when that happened the next relapse occurred after double the usual apyrexial period for that particular case. Some patients had as many as seven relapses. Complications were iritis, jaundice, lobar pneumonia, cerebral disturbances (including coma, hemiplegia and cerebral nerve palsies, with or without pleocytosis in the cerebrospinal fluid), and severe bronchitis, in that order of frequency. Iritis was not a presenting symptom and did not occur with the first pyrexial period. Cerebral palsies sometimes came on quite late in the disease. At no period did the case mortality ever rise as high as 1 per cent.

In controlled experiments, novarsenobillon appeared to be of little or no value in treatment of this tick borne type. Incidentally competent observers in Addis Ababa told me they were even doubtful of its value in their house-borne variety. Penicillin was also tried: it had some effect, but the results were not sufficiently good to justify its general use.

Relapsing fever was uncommon among Europeans when it did occur cerebral complications were seen much more frequently than in Africans.

#### SCHISTOSOMIASIS.

The small number of non Europeans admitted to hospital for schistosomiasis gives no idea of the real incidence of this extremely common infestation in the East African native. It does illustrate how often the African either ignores or is quite unaware of his disease. Some idea of the prevalence of *haematobium* schistosomiasis, which is much more common than *mansoni* schistosomiasis in East Africa, may be gained from Table IV which gives the results of the examination of the urines for ova of 1 408 African troops, all of whom were in active training and apparently fit. Many other similar series were examined. This particular one was done by DRANSFIELD (1944). It will be seen that by far the highest percentage of the men affected came

from Nyasaland. In that country there is a large lake, and it is probable that all the rivers running into it from the surrounding mountains and the pools of water lying behind the foreshore, are infested along the greater part of its length.

The population on the lakeside is heavy, and it is thought by local observers that 90 per cent. of the inhabitants have the disease from early childhood.

Rectal schistosomiasis appears to be relatively uncommon although the distribution of *Schistosoma mansoni* throughout East Africa is not at all accurately known. Fortunately, the incidence of both types of disease among Europeans, in whom the symptoms can be so troublesome, was very small.

TABLE IV

EXAMINATION OF THE URINE FOR OVA OF *Schistosoma haematobium* OF 1408 AFRICAN TROOPS, ALL OF WHOM WERE IN ACTIVE TRAINING AND APPARENTLY FIT

	Number of men examined.	Percentage with ova in urine
Nyasaland troops,	351	41.59
West Nile troops	228	7.89
Tanganyika	546	2.80
Other tribes including Kenya and Uganda	303	2.31
All troops	1,408	13.56

#### MENINGITIS

Relative to European troops acute pyogenic meningitis was common in African troops and had a very severe clinical course. Towards the end of the war the incidence had fallen but the prognosis remained as grave. Most of the fatal cases were comatose, or semi-comatose, on admission and died in spite of prompt diagnosis and treatment. Usually the infection was meningococcal sometimes it was pneumococcal, without any local focus being found elsewhere in the body. Some of these pneumococcal cases were particularly resistant to treatment, both with sulphonamides and with penicillin.

#### DENGUE.

The garrison town of Diego Suarez, at the north end of Madagascar was until recently a hot bed of dengue during the early months of the year. Thus from January to the middle of May 1944 the number of patients suffering from dengue admitted to the military hospitals of Diego Suarez was 94.2 per cent. of the total number admitted to all military hospitals in the Command during the whole year. Europeans were attacked over eight times more



## NUTRITIONAL DEFICIENCY DISEASES.

Although there was plenty of evidence of malnutrition in recruits (and these have been ably studied in the last few years by KEKWICK, WRIGHT and others (1946), florid examples of nutritional deficiency were rare. However, in March, 1945 there was a sharp outbreak among East African troops in Diego Suarez of a syndrome indistinguishable from beriberi, characterized by oedema of the legs, peripheral neuritis, and right-sided dilatation of the heart. In November 1944 it had become necessary in Diego Suarez to substitute rice for maize meal as the staple article of food in the African diet. By the middle of February 1945, for various reasons, other articles of food were in short supply and the main part of the African diet then consisted of 19 ounces of rice daily. In March the syndrome appeared.

The rice had been lightly milled and in its uncooked state contained a theoretical sufficiency of thiamin and was active and adequate in relation to pigeons.

After we had examined various possibilities it was found that while fresh, lightly milled rice lost only 30 per cent. of thiamin in cooking the suspected rice, which was old and contaminated, lost over 70 per cent., and, moreover, produced beriberi in pigeons (CULLENAN, KEKWICK, WATTS and TITMAN (1946). It is possible that similar outbreaks may exhibit the same aetiological sequence of a toxin acting upon the thiamin and producing an epidemic of beriberi.

## TUBERCULOSIS.

In general, it may be said that pulmonary tuberculosis among young adult East African natives is represented by the acute types of the disease. The diagnosis is often not easy—the disease, which frequently runs a severe course, may be far advanced when a patient is first seen—the death-rate is high. The majority of Africans suffering from tuberculosis have pulmonary involvement. Frequently extensive lesions are found in the lungs of patients who have complained of only a few days' illness, or the disease is first revealed by a seemingly innocent acute respiratory infection. Some run an acute and rapid course to death. The disease may simulate other diseases and the diagnosis first be made on the postmortem table. The lesions at postmortem were usually extensive and bizarre. But there is a group with higher resistance which responds well to treatment. It was mainly for this reason that a special chest centre was formed in the Command. This was under the charge of C. S. DAKKE. The basis of treatment consisted of good food, freedom from fatigue without strict bed-rest, and where possible artificial pneumothorax. An appreciable proportion of African patients thus treated very greatly benefited from such treatment, and—what is most important—the treatment was looked on with favour by the Africans. Unfortunately most of the patients, because they had been away from their kinsfolk for a long time, and particularly because they felt better as a result of treatment, were unwilling to stay in hospital longer than 3 months, and wanted to join their families in the reserves.

It is a widely accepted belief that the resistance of natives to infection is not so great as that of the white races. According to WILCOCKS (1938) the resistance lies midway between the completely primitive races and the relatively resistant European and, further, the most important factor in the spread of the disease is contact with sputum positive cases, which is closest in families and which outside families varies directly with the density of the population.

The civil governments are thus faced with a problem, of the gravity of which they are fully aware. How are they to stop the spread of the disease? The African refuses to be separated from his family. The 'settlement' system is one possible answer and Kibongoto a tuberculosis centre on the lower slopes of Mt. Kilimanjaro in Tanganyika, is doing excellent work in this direction.

I must leave consideration of the many other fascinating disorders, including respiratory diseases and the anaemias, and go on to the second part of the paper.

## II THE NEED FOR CO-ORDINATED CLINICAL INVESTIGATION IN THE FUTURE

(including reference to the Medical Investigation Committee,  
East Africa Command)

Today, research is well recognized to be a prime essential for progress by all planners of the New World. In medicine, it is the means of all future advance. But the phrase medical research covers a large variety of methods ranging from complex and highly specialized investigations requiring the aid of up-to-the minute scientific technique to simple clinical observations. It is worth while pausing to consider what methods are most applicable to the study of disease in parts of the British Colonial Empire such as East Africa. In Britain itself methods have of necessity veered more and more towards highly specialized investigation. Clinical observations have at the moment yielded apparently all they can and the simple direct methods of SYDENHAM and MACKENZIE are said to be out dated. But whether or not this is true for Britain, it is certainly not true for East Africa. There, in those huge territories where diseases, familiar and unfamiliar attack many races of peoples living in different conditions of climate and nutrition, the necessity and opportunities for simple clinical observations are still great. Fundamental basic problems affecting health and disease have not yet been worked out. Their solution is not only vital for the progress of East Africa but might well shed new light on the spread and natural history of disease. Nor are the problems by any means confined to tropical diseases. For instance, what is the extent and distribution of tuberculosis in the Territories, how do its effects differ in the native and in the European how much tuberculosis is bovine? Is the native liable to rheumatic infection, and how often, and with what effects?

Why are gastric diseases uncommon? What is the incidence and aetiological situation of carcinoma? And so on, through the gamut of medical disorders.

These problems demand an answer. Much valuable work has already been done by individual workers but to get anything really big out of the future careful planning and co-ordination will be required. Plans for post-war research in the Colonial Medical Service are already being prepared and it is earnestly to be hoped that the great need and scope for these clinical investigations will be realized to the full. Such investigations cannot be confined only by one or two men working in a laboratory although these may be important ancillaries—they require large numbers of observers scattered out and about in the Territories who are all working to a unified plan.

The time is opportune to describe briefly a start in this direction that was made during the recent war by the Army Medical Services in the East Africa Command. As a newcomer to this great area, it did not take long to realize that innumerable clinical and epidemiological questions which confronted the Medical Services invited investigation, and that a number of investigations had been made and had furnished conclusions of value. More, however, had to be abandoned because of the exigencies of the Service and still more had never been undertaken because of the uncertainty of bringing them even to an interim conclusion. After several of us, who subsequently became members of a Medical Investigation Committee had discussed this, we felt that the only way in which continuity could be secured was by means of some central co-ordinating body.

Such a body by narrowing each investigation to specific limited objectives, by dividing investigations into sections to be dealt with by different observers or groups of observers, and by holding records and maintaining the general plan of a particular investigation, could ensure the continuance of a set of observations. It could, moreover give advice and assistance in the planning of work, and in dealing with difficulties which might arise during its course. It could also assist in providing materials that might be required, and in collecting particular types of case in particular localities.

Thus it was that in June, 1944 the Deputy Director of Medical Services (Brigadier R. P. COMBICK) inaugurated a Medical Investigation Committee for the purpose of encouraging, facilitating and correlating research by officers of the Medical Services in the East Africa Command. Its aims were made known to all officers. The response was immediate and enthusiastic, and although the scheme had been going for little over a year before V.J. Day the fruits were already beginning to ripen. Completed investigations, the

\*The members of the Medical Investigation Committee were Brigadier E. R. COLLINAN (Consulting Physician), Colonel T. F. ANDERSON (then, Assistant Director of Hygiene), Colonel D. D. MCCARTHY, Lieut.-Colonel D. BACOTTE WILSON (Malariologist), Lieut.-Colonel A. KIDWELL and Lieut.-Colonel P. E. C. MASON-BURN (Officers in charge of Medical Divisions), Major G. W. DECK (Parasitologist), and Major A. S. HOCKING (Entomologist). Lieut.-Colonel D. BACOTTE WILSON is now Chairman.

results of which have been published or recommended for publication, include A Study in Nutrition of East African Army Native Personnel, KEKWICK and WRIGHT (1946), Malaria in Abyssinia, MELVILLE, WILSON and others (1945), Ascorbic acid metabolism in East African Army Native Personnel, KEKWICK, RAPER and WRIGHT (1946) Datura poisoning ANDERSON and others (1944), and a Description of an Outbreak of Beriberi with special reference to a discussion on the aetiology of beriberi and epidemic dropsy, CULLINAN and others (1946)

Several other investigations are nearly finished, including various problems of Psychoneurosis in Africans Tuberculosis in Africans Schistosomiasis—tribal survey and criteria of diagnosis and cure Relapsing Fever in Africans—complications, and assessment of treatment Thrombophlebitis in Africans, A Typhus-like syndrome

A number of other investigations are in progress.

Recently a mobile pathological laboratory was formed complete with transport and equipment. This is a valuable unit for these types of investigations as it is able to move about the territories quickly and thereby bring pathological assistance to the doorstep of any particular enquiry

Now the war is over it will be impossible for that work to be continued by the Army Medical Services in East Africa.

This scheme for medical investigation has been described at some length not to advertise its wares but in the hope that members of the Colonial Medical Services will be interested in it. If they were to carry on with a similar scheme, and develop it their scope would be far greater than ours could ever be. They would neither be hampered by frequently changing personnel nor limited to problems primarily of military importance, nor bound to a short term programme To get full value and unity of purpose, it would be necessary, of course, for such a scheme to embrace all East Africa and not be confined to a single colony where the scope would be much too narrow

Apart from its potential scientific value, it has in the Army another great advantage namely that large numbers of medical officers can take part and be interested in it. From past experience in remote stations I know of little which more successfully wards off the ever-present danger of ennui. Surely this would apply just as much to members of the Colonial Medical Service many of whom, keen and hard working are stuck in out-of-the-way places for long stretches of time with no medical contacts and small chances of an early move.

These suggestions are not intended to decry the value of highly specialized investigations in East Africa, but to stress the fundamental need for widespread co-ordinated epidemiological and clinical observation of basic medical problems in addition to more eclectic research. The field is great and most promising for study unlike the field at home about which so much is already known.

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## DISCUSSION

Dr A. Kekwick. I would like to associate myself particularly with Dr CULLINAN's remarks about clinical observation and research in East Africa. After 3½ years there it seems to me that the picture is exactly as he portrayed it—that there is an incredible amount of accurate clinical observation and simple recording to be done before the time is right for using highly organized and specialized research techniques. For example the normal range of blood pressure in natives has not been estimated—we ourselves did five hundred, and it is obvious that thousands more should be done. In my view this method of investigation particularly applies to nutrition. The clinical manifestations of malnutrition are very little seen in this country but in Africa they play an important part in the clinical aspects of every disease.

There are also a large number of rare diseases in East Africa which offer tremendous facilities for observation and research in themselves. Such things as sudden inexplicable cardiac failure of a peculiar type—conditions like one of which we saw much where there is intense neck retraction, normal cerebrospinal fluid and recurrent pyrexia, and finally an epidemic form of thrombophlebitis.

Such diseases are not only interesting in themselves but their study would probably add materially to our knowledge of the mechanisms of medical conditions occurring in England.

Dr C. Wilcocks. I was much interested in Dr CULLINAN's reference to tuberculosis. Some of us, including Colonel S. LYLE CUMMINGS and Sir LEONARD ROGERS, have, for many years, been preaching tuberculosis in the tropics, and have felt that this disease in the indigenous populations has not received the attention it has deserved in the past and will probably deserve to a much greater extent in the future. I do not know what is the answer to this problem, but it may be something like the answer to many of the other diseases from which the Africans suffer. The impression

one obtains in Africa is that, apart from the actual pathological agents, the fundamental origin of many diseases lies in poverty and ignorance. It is to be hoped that, whatever organization may be created to undertake the investigation of disease in East Africa and elsewhere, it will set itself as one of its main objects the task of impressing upon the Governments the importance of these factors in the fundamental aetiology of disease. It is a duty of doctors throughout the world to insist that the economic and administrative policies of Governments affect the health of the people—as is recognized in the recently published West India Royal Commission Report—and that they must be considered from this point of view.

In small out stations in the tropics there are excellent opportunities for clinical investigation and for study of the gross pathological changes connected with diseases in general. I found it quite easy on such a station to perform a series of postmortem examinations and from them, though not a skilled pathologist, I formed a clearer idea of what was going on inside the body of the African.

Str Phillip Manson-Bahr I have listened to this paper with a great deal of delight and satisfaction, because it seems to me that what Dr CULLINAN has said has presented us with new aims in many directions. First of all he stressed the importance of clinical observation as part and parcel of the daily life of the colonial medical officer. It seems to me that we have now reached the parting of the ways with regard to our Colonial Medical Service. This has been the subject of a certain amount of criticism, and it is time we should think—and this Society has already started to think—how we can help in research so as to make the work of the colonial medical officer bear more fruit. This service is not so popular as it ought to be because of the fear on the part of young men that they will be sent to some isolated station far removed from contact with their professional brothers, with no opportunities of advancement in the medical sphere. They dread lest they be reduced to the status of clerks signing papers and issuing returns and this sterilizes research work such as many of these men could perform. A scheme such as Dr CULLINAN outlined should be brought to the notice of the medical authorities at the Colonial Office. We know that plans are being formed on which the future of the Colonial Medical Service should be based. One of the plans that has been envisaged has been that of creating an Empire team of research workers who would have intimate contact with the Medical Research Council and other bodies in this country and who would be able to despatch by air those skilled in the investigation of certain diseases to do the laboratory part of such work. Such a team is easily constituted and as has recently happened has been flown to Mauritius to investigate outbreaks of infantile paralysis. Thus they could obtain most valuable data and material. That is one part of the idea Dr CULLINAN has given us tonight. The other is the encouragement of pure clinical observations—the sort that he has inculcated in his students at St.

Bartholomew's Hospital on the basis of clinical medicine, which most doctors are capable of carrying out and of which too little has been done in the tropics. (Too much emphasis, it seems, has been laid on the purely laboratory side of medicine in the tropics.) Observations of great importance can still be made in this wide open field. I have been impressed all my life by the different appearances of the same disease in different races—their reactions to pneumonia, tuberculosis, cerebrospinal meningitis and many others. For instance, there is the similarity of sandfly fever to dengue. Dr CULLINAN mentions certain diseases in his survey but he did not mention sprue. I presume he did not see it. I have recently read a very fine paper from Lt. Col. LEISHMAN who has told us about sprue in India where it has been behaving almost as an epidemic disease. Why is it absent from West and Central Africa? To my mind there lies the probable explanation of the genesis of sprue, if we can find out what that means. There are almost limitless questions to which the answer can be given by ordinary clinical observation.

**General A G Biggam** I would like to say how much I have enjoyed Brigadier CULLINAN's talk this evening. He has shown what an advantage it is to have a new mind directed on some of our old tropical problems. I would like to congratulate him on his excellent paper.

**Brigadier J S K Boyd** I can give the Society some news on the subject of medical research in the colonies. The Secretary of State for the Colonies has already formed a Colonial Medical Research Committee under the Chairmanship of Sir EDWARD MELLANBY. Large sums of money are available for research purposes and a Director of Medical Research is about to be appointed. It is proposed that there should be Scholarship funds available for training selected research workers, and that, probably as a branch of the Colonial Medical Service, there should be whole time research workers controlled by the Colonial Medical Research Committee. The work will be on the widest possible basis, covering pathology, medical entomology, helminthology, protozoology, nutrition, general medical problems such as tuberculosis, and so forth. The Colonial Medical Research Committee will maintain liaison with the Colonial Agricultural Research Committee, the Colonial Social Service Research Council, and other bodies linked with a central Colonial Research Committee. I think we can look forward to important developments along these lines in the near future.

**Colonel S P James** said that in listening to Dr. CULLINAN's interesting paper he was surprised to hear again the opinion that nearly all the malaria in East Africa is caused by the malignant tertian parasite. This opinion was expressed by observers in 1899-1900 but he thought that the considerable prevalence and importance of other species than *falesparum* had been recognised for many years. He was sure that Dr. LAUTZKE, if he were present, would recall the frequency with which we found quartan parasites in the blood of

native children in 1929. It is also well known that relapses of quartan malaria occur frequently in Europeans home on leave from East Africa. A high incidence of *vovax* malaria and a lesser incidence of *ovale* malaria was also widely recognized, and he thought that Dr GARNHAM and others had drawn attention to the possibility that the classical *falciparum* of Europe may not be the only malignant species of parasite present in the country. Moreover the notable researches of Dr and Mrs. BAGSTER WILSON on malarial infection in native babies and young children have shown clearly that the true picture is an almost continuous infestation with several species, of which one or other becomes dominant from time to time. The symptomatology and pathological effects of this prolonged parasitic infestation would be, of course a very proper subject of study by the Clinical Research Organization to which Dr CULLINAN referred. Unfortunately, it is not an investigation that can be satisfactorily conducted in hospitals. As Dr BAGSTER WILSON has so ably shown it can be done only by enthusiastic individual medical officers whose other duties allow them time and opportunity to get in close touch with particular native families in small villages, to win their confidence and co-operation in the enquiry and to follow the daily life and medical history of each member of the family for a long period.

Dr G Carmichael Low: I do not know if Dr CULLINAN saw cases of trypanosomiasis amongst the troops. He did not say where the natives came from, probably none were from endemic areas. I was very interested in the different diseases mentioned as they were much the same as those I saw more than 40 years ago now when I was with the first Sleeping Sickness Commission in Uganda.

What struck me in those days was the number of diseases an individual might have and still survive. At the autopsy of sleeping sickness cases, one found evidence of old malaria, pigmented spleens, dysenteric ulcerations and many helminthic infections. Bilharzial lesions of the bowel and liver due to *Schistosoma mansoni* were common as were also ankylostomiasis, ascariasis and filariasis due to *Acanthocheilonema perstans*.

Skin diseases and malnutrition prevailed and syphilis and yaws were widespread. Tick fever very often associated with iritis, occurred in several of the white inhabitants. Dr CULLINAN no doubt, did not have time to go into the question of filariasis—*Wuchereria (Filaria) bancrofti* is very prevalent among the natives of the coast of East Africa, and when further research is carried out this subject should not be neglected.

Mr McKim McCullagh said he investigated the relative frequency of appendicitis amongst the blacks and whites of West Africa and found only one case in natives to thirty amongst the whites. This preponderance of appendicitis amongst the whites was therefore intense as there were relatively few white troops in proportion in West Africa. He wondered whether the



same preponderance occurred in East Africa. He felt some difference in *set* was the cause of this great difference in frequency and that the same factor would explain the eight times greater frequency of appendicectomy amongst the public school boys of Britain as compared with the boys at elementary schools.

Dr L. E. Napier May I add one remark. I noticed that each of the first two speakers, Dr CULLINAN and Dr HEKWICK, both of whom have done most of their work in this country emphasized the value of tropical studies on the advancement of our knowledge of medicine generally of cosmopolitan medicine. Many of us are engaged in studying so-called tropical medicine, but it cannot be emphasized too frequently that the scientific knowledge that is gained in these studies will frequently be reflected in the practice of medicine as it occurs in this country. This is my plea for a wider interest in tropical medicine.

Dr Cullinan (in reply) Colonel JAMES asks the incidence of quartan malaria. Very few cases of quartan malaria were reported among the troops in the territories except from Mauritius. Intis was the most common complication of relapsing fever. I have given the figure as 17 per cent. but this is probably too low. Dr HEKWICK, who was in charge of the medical division of a large African hospital found the complication in over 20 per cent. of patients suffering from relapsing fever. The difference in the types of psychoneuroses affecting Africans and Europeans is very interesting. The African tends to suffer from hysteria the European from anxiety states. Anxiety states are rare in Africans, and when they occur do so in those who have been educated beyond their fellows. Brigadier BORD points out that pneumonia was common among Africans in the Middle East. This is true of Africans in East Africa, although, surprisingly the ratio of Africans and Europeans affected was only 100/100. Dr CARMICHAEL LOW refers to trypanosomiasis. At the time I was in East Africa there was no trypanosomiasis in the Army although it had been there earlier.

Concerning schemes for future colonial research, my plea is that these schemes should include organized clinical investigations by the medical officers on the spot, as well as more specialized investigations carried out by selected teams. The medical officer in the Colonies who has the time is only too keen to get his teeth into this type of work, but unless his work is organized and there is somebody to bring him into contact with others interested in it, he can have little incentive to carry on. There are, of course, brilliant exceptions. If there were some scheme by which everybody could be encouraged to work together I am perfectly certain that very large results would accrue. A concerted study of medicine in the tropics would not only advance knowledge of tropical disorders, but, as has been ably said in the discussion, might well shed new light on the process of ordinary non-tropical disease.

## COMMUNICATIONS

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### MITES (ACARINA)—A PROBABLE FACTOR IN THE AETIOLOGY OF SPASMODIC BRONCHITIS AND ASTHMA ASSOCIATED WITH HIGH EOSINOPHILIA

BY

HENRY F CARTER

AND

V Sr E. D ABRERA.\*

*Division of Medical Entomology, Department of Medical and Sanitary Services Ceylon*

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During the past few months research in relation to the occurrence of mites in human sputum (CARTER, WEDD and D ABRERA 1944) has been confined largely to the study of cases of respiratory disorders chiefly bronchial asthma, associated with high eosinophilia. This paper gives the results of investigations conducted on twenty-five Ceylonese patients all of whom were suffering from—or had recently suffered from—respiratory disorders, and none of whom showed an initial eosinophilia of less than 3 000 per c.mm. Eleven of the cases were under observation and treatment at local hospitals.

In some of the cases the clinical, radiographic and haematological pictures conformed to those described by recent authors as characterizing the condition variously specified as Pseudo-tuberculosis (FRIMODT-MÖLLER and BARTON 1940) Tropical eosinophilia (WEINGARTEN 1943) and Eosinophil lung (CHAUDHURI, 1943) but in others the clinical or radiographic features of the syndrome were modified, and in one case (Case 21) the subject was in good health at the time of examination save for a massive eosinophilia.

\*We are much indebted to Surg. Lt. Cmdr G WEDD R.N. for his collaboration during the early part of these investigations and for supplying us with full details in respect of the histories, blood examinations and radiographic findings of Cases 10, 13 and 16. We are also indebted to Lt. Col. F. G. SMITH, C.M.C., Major E. SOYSA, C.M.C. and Capt. M. D. S. JAYAWARDENE, C.M.C. for data regarding Cases 5, 11 and 12 to Drs. D. J. T. LEANAGE, V. E. P. SENEWRATNE and E. M. WIJERAMA for directing our attention to several cases at the General Hospital, Colombo and to Dr. J. A. SIRIWARDENA for referring us to Case 17. Our thanks are also due to Mr. J. V. COLLINS, O.B.E., Government Analyst for the examination of the arsenic content in the sample of sputum referred to on page 385 and to Mr. P. G. JAYARATNAM, laboratory assistant, for much help in the preparation and examination of specimens of sputum for mites.

Circumstances, unfortunately did not always permit observations to be made as consistently or extensively as was desired and in several cases radiographic examinations were not possible and blood and sputum records were limited. Selection of a case for investigation in this series was made dependent, primarily upon the results of an initial examination of the blood. When the eosinophil count was high, 24-hour samples of sputum were collected and examined for mites. Treatment with organic arsenicals—usually stovarsol—was then given and specimens of both blood and sputum examined whenever practicable.

### CASE HISTORIES.

Brief particulars of all the cases investigated are included in Table I, p. 388 but fuller details of six cases of special interest are given below.

#### *Case 1—J. R. Schoolgirl aged 10 years*

Chronic bronchitis and emphysema. From August, 1939 to December 1941 admitted to various hospitals where typhoid, malaria, bacilluria and tuberculosis were excluded by the usual pathological tests. Treated for hookworm in December 1941. In May, 1942, a differential count showed an eosinophilia of 52 per cent. A diagnosis of bronchial asthma was made and she was treated with M. & B. 693 (thirty tablets over a period of 8 days), whilst for the urinary disorder mandelic acid therapy was prescribed. On 26 June, 1942, as no improvement in her condition had occurred she was readmitted to hospital and submitted to the usual pathological tests, all of which proved negative except that of the urine which showed *B. coli*. An autogenous vaccine was prepared and administered. The skiagram of the chest showed chronic bronchitis. Tonsillectomy was performed on 14th March, 1943. In December 1943 our attention was directed to the case, and on examination of the blood a leucocyte count of 41,000 per c.mm., with eosinophils 23,800 per c.mm. (70.3 per cent.), was found. Samples of sputum and urine were then examined for mites. Six 24-hour samples of sputum—collected only with difficulty and by means of close personal attention since the patient usually swallowed her sputum—were obtained between 7th and 14th December. Mites were present in three of these samples. During the same period five samples of urine taken at 8 a.m. after withholding all fluids from 8 p.m. the preceding evening and voided directly into prepared flasks after ablation of the parts—were examined and mites (total twenty-five specimens of various species) were found in every sample.

Treatment with stovarsol was given from 14th to 21st December 0.15 grammes thrice daily for the first 3 days and once daily thereafter. Eight samples of sputum and two of urine were examined during treatment, three of the former and one of the latter containing mites. By the end of the treatment period the sputum had diminished and the cough had disappeared and although fluids in liberal quantities were allowed no course occurred. On the last day of treatment the leucocyte count was 40,000 per c.mm. with eosinophils 30,680 per c.mm. (76.7 per cent.) but 2 months later (after a second course of stovarsol extending from 8th to 10th January inclusive total 0.65 grammes) the count was 31,800 per c.mm. with eosinophils 2,300 per c.mm. (19.5 per cent.). In December 1944 the parents stated that the child was free from all symptoms and was attending school.

#### *Case 3—K. G. P. Male aged 28 years*

Cashier in small bakery. Admitted to civil hospital 28th December 1943, with history of spasmodic cough and low fever of 8 months' duration. Physical examination revealed rhonchi and rales over both lungs. Radiography showed "evidence of mottling of both lungs—eosinophilic lung. Sputum examined for tubercle bacilli, negative. W.B.C. 24,000 per c.mm. eosinophils 12,000 per c.mm. (50 per cent.). On 2nd January 1944,

developed typical asthmatic attack which lasted until the following day and was controlled by ephedrine and adrenalin. On 6th January the blood sedimentation rate (Westergren's method) was 35 mm. for the 1st hour and 64 mm. for the 2nd hour. No malaria parasites were seen in the blood and no amoebae or amoebic cysts in the stools which, however, contained a few hookworm eggs. Samples of sputum were examined from 29th to 31st December 1943 and mites (seven) were found in two out of three samples. Treatment with stovarsol was carried out from 3rd to 8th January 1944 (3.12 grammes) and again from 20th to 22nd January (2.08 grammes). During the first course of treatment six 24-hour samples of sputum were collected and mites were found in three—twenty mites of five different types being present in the sample obtained during the first 24 hours of treatment, ten mites in the second sample and one mite in the fifth sample. Following the completion of the second course of treatment, three further samples of sputum were examined: a single mite was found in one of these. No other samples of sputum were obtainable: the lung condition cleared and asthmatic attacks ceased. The sedimentation rate on 3rd February was 8 mm. for the 1st hour and 21 mm. for the 2nd hour. The final blood examination, made on 29th July gave a leucocyte count of 11 000 per c.mm. with eosinophils 1 100 per c.mm. (10 per cent.) on which date the patient stated that he was well and had had no further attacks of asthma.

#### Case 10—F. Male aged 20 years

Naval rating. Suffered from asthma intermittently over a period of 7 years, the present series of attacks (two or three short, sharp attacks each month) having commenced in October 1943. Admitted to hospital on 26th February 1944. Physical examination revealed prolonged expiration with moist sounds over both lungs: three examinations of sputum for tubercle bacilli, negative. Blood count, total leucocytes, 25 000 per c.mm., with eosinophils 17,375 per c.mm. (69.5 per cent.). Stools contained ova of *Trichuris*, *Ascaris* and *Ancylostoma*. Two 24-hour samples of sputum taken on 3rd and 4th March were examined and a single mite was found in the former. Treated with chenopodium, carbon tetrachloride and santonin and examination on 7th March, showed stool free from worm ova. On 6th March the patient developed an attack of asthma and from 8th to 10th March he suffered from several attacks of expiratory dyspnoea. Blood examination on 12th March gave a leucocyte count of 30 000 per c.mm. with eosinophils 18 450 per c.mm. (61.5 per cent.). Skiagram on 19th March showed a marked increase of striae, fibrous emphysema and gross and fine mottling over all areas of both lungs and an ill-defined area at the third sternal interspace suggestive of cavitation: appearances suggestive of asthma, although TB cannot be excluded.

Treatment with stovarsol, 0.26 gramme twice daily for 10 days, was commenced on 12th March, and during this period seven 24-hour samples of sputum were examined. A total of 24 mites of which 15 were a species of *Tarsonemus* was recovered for the first three samples, the others being negative. On 12th and 13th March the patient suffered from mild attacks of asthma but subsequently remained free: the chest condition was clear by 20th March. Blood examinations made on 27th March and 24th April, 1944 gave respective counts of total leucocytes 10 000 and 6 700 per c.mm. and eosinophils 2,800 (28 per cent.) and 400 per c.mm. (6 per cent.).

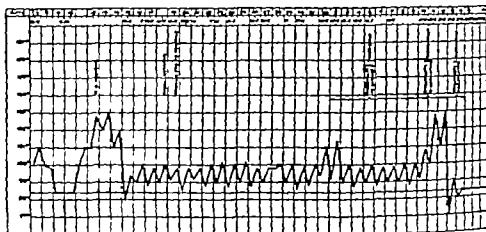
#### Case 17—M. Male aged 57 years

Planter. On 7th May 1944 developed fever, cough and attacks of dyspnoea. Malaria was suspected but no parasites were found in the blood. Between 12th and 23rd May standard agglutination for typhoid, examination of sputum for tubercle bacilli and examinations of stools and urine were carried out with negative results, and a differential blood count made on 16th May showed an eosinophilia of 68.1 per cent. Asthmatic attacks increased and adrenalin injections (10 minims twice daily) were given from 2nd to 8th June. On the latter date a 24-hour sample of sputum was examined and a single mite of an unidentified species was found.

Treatment with stovarsol in doses of 0.26 gramme made up in cachets with nicotinic acid (20 mg.) and calcium lactate (10 grains) was commenced on 9th June and continued until 15th June, a total of 2.6 grammes of stovarsol being administered. On 11th June

a mite (*Tyroglyphus*) was present in the sputum. On this day the patient's condition deteriorated, the temperature rose to 103° F (see chart) and status asthmaticus supervened, but treatment was continued and on 13th June the asthmatic attack ceased completely and the temperature fell to 97° F. On 31st July blood examination gave total leucocyte count of 8,000 per c.mm. with eosinophils 1,950 per c.mm. (24.3 per cent.)

TEMPERATURE CHART Case 17



## Case 18.—P. S. Male aged 33 years.

Engineer. History of intermittent bronchial asthma since the age of 9, the periods of subjection to, and freedom from, attacks sometimes being as much as 2 or 3 years. The present series of attacks commenced in August, 1942. On 30th March, 1944, the total leucocyte count was 17,600 per c.mm. with eosinophils 9,500 per c.mm. (53.2 per cent.) and on 25th May the leucocytes were 24,000 per c.mm. and eosinophils 11,900 per c.mm. (58.2 per cent.). From 26th May to 5th June nine 24-hour samples of sputum were examined and three mites were found in three of them.

Treatment with stavarsol was commenced on 6th June, but was discontinued on 8th June as the asthmatic condition on 7th June was exacerbated (temperature 100° F.), and on 8th June status asthmaticus supervened, the temperature rising to 105° F. while the chest signs were typical of bronchopneumonia. Adrenalin (10 minims, subcutaneous) and M. & B. 693 (as 1 gramme doses every 4 hours, total 5 grammes) were administered. From 9th to 11th June the patient's condition improved rapidly and the temperature fell to normal. Treatment with stavarsol was resumed from 20th to 23rd June the total quantity given during the interrupted course being 2.66 grammes. Between 6th and 17th June four 24-hour samples of sputum were examined but no mites were present. A second course of treatment, using carberapoe, was administered from 17th to 24th August. A final blood count made on 26th October 1944 showed leucocytes 9,200 per c.mm., with eosinophils 900 per c.mm. (9.8 per cent.). On this date the patient was in good health—he stated that he had had no attack of asthma since 9th June 1944.

## Case 25.—A. de S. Male aged 51 years.

Stores clerk. Asthma commenced in June 1927 a few months after his appointment to the stores, and since that date he suffered severely. Several proprietary preparations for asthma were tried, but none gave more than temporary relief and he was obliged to retire from work prematurely a few years ago. When first seen by us in August, 1944, he was in a distressed condition following a climb of a short flight of stairs to the laboratory. On 3rd August a blood count gave total leucocytes 32,000 per c.mm. with eosinophils

18,400 per c.mm. (57.5 per cent.) Between 4th and 8th August five 24 hour samples of sputum were examined and mites were found in two of them.

Treatment with stovarsol was carried out from 9th to 18th August, a total of 3.9 grammes being given. 50 mg. of redoxon were given with each tablet of stovarsol. During the period of treatment five 48-hour samples of sputum were collected and examined: five mites were present in the first two of these samples, the remainder and two other samples taken after treatment was completed, being negative. Since the asthmatic condition had not entirely disappeared, a second course of stovarsol (total 1.56 grammes) was administered from 28th to 31st August. The final blood count taken on 10th October 1944 showed total leucocytes 11,000 per c.mm. and eosinophils 830 per c.mm. (8.5 per cent.) On this date the patient stated that he had been free from asthma since the end of August and that his sleep was undisturbed—a boon that he had not experienced for many years. His lungs were clear and no dyspnoea occurred on exertion.

### EFFECTS OF TREATMENT ON THE CLINICAL CONDITION

As previously indicated organic arsenicals were employed in all cases, stovarsol 0.26 gramme tablets being used in twenty-one cases, carbarsone 0.25 gramme tablets in three cases (Nos. 12, 22 and 23) and novarsenobillon in one case (No. 19). The oral method of administration was preferred to the parenteral since it precluded the preparations needed for the latter and also limited the period of treatment to at most 12 days, whereas the parenteral method would have involved approximately 4 weeks (two injections per week). In five cases (Nos. 20 and 22 to 25) the arsenical was combined with vitamin C (redoxon) in 50 mg. tablets—one tablet of the latter being given with each arsenical tablet. The administration of this vitamin as a precaution against the possibility of intolerance to arsenic was indicated by the work of DAINOW (1937) and LAHIRI (1943).

Of the twenty five cases treated with arsenicals one only did not respond satisfactorily. In this case (No. 15) a clinical relapse occurred one month after completion of treatment although the eosinophilia had fallen from 3,000 to 920 per c.mm. The rest have shown no recurrence of clinical symptoms up to the time of writing (November 1944, *i.e.* from 4 to 11 months since the completion of treatment) and in all of them the eosinophilia was greatly reduced.

Although GRENIER (1924) considered the use of stovarsol in the treatment of amoebic dysentery safe, eight of the cases in this investigation suffered from exacerbation of the asthmatic condition following administration of the drug. In five of these cases the degree of exacerbation was moderate and was controlled, while pressing the arsenic treatment by the administration of a powder containing ephedrine hydrochloride ( $\frac{1}{4}$  grain) phenacetin (3 grains) and caffeine citrate (2 grains) in two doses at an interval of 4 hours. In the remaining three cases however the condition was such as to cause anxiety. In Case 17, the patient's condition became grave, but continued treatment with stovarsol resulted in a dramatic cure comparable with that described by PARSONS-SMITH (1944) following the use of neoarsaphenamine intravenously. In Case 18 stovarsol was temporarily discontinued and the superimposed pneumonia treated with V.L. & B. 693. It is not possible to state whether this pneumonia

was due to an invasion by a pneumococcus or streptococcus as no culture was made but it is of interest to note that no response was obtained when M. & L. 693 was used in Cases 1 and 5 for treatment of the asthmatic and eosinophilic conditions. In this connection attention may be drawn to the isolation by ALWALL (1943) of pneumococcus (Types 7 31 and 33) in two cases of pulmonary infiltration with eosinophils—Loeffler's syndrome—and to the satisfactory results obtained by treatment with sulphathiazole. In Case 11 the patient developed an acute renal colic on the 3rd day after completion of treatment and was removed to hospital where she became unconscious the following day and remained so for 48 hours. It may also be mentioned that in another case with an initial count of 3,100 eosinophils per c.mm.—not included in the series as no suitable arrangements could be made for collection of sputum—where the patient was treated with stavarsol (total 3.9 grammes over 11 days) and vitamin C she developed fever and later on the 3rd day after completion of treatment, became unconscious she remained in a comatose condition for 4 days, when sodium thiosulphate was administered and consciousness was regained almost immediately.

#### HAEMATOLOGICAL FINDINGS.

The results of blood examinations before treatment with organic arsenicals and at various dates after the completion of treatment, are given in Table II. Both absolute and differential leucocyte counts were made in twenty-three of the twenty five cases under review. The differential counts were made on a minimum of 500 leucocytes.

In the twenty three cases for whom absolute counts were made the total white blood cell count prior to treatment ranged from 9 000 to 53,200 per c.mm., and exceeded 20 000 per c.mm. in seventeen cases. The eosinophil count in these cases prior to treatment varied from 3,000 to 42,550 per c.mm., and exceeded 10 000 per c.mm. in sixteen cases.

For ease in comparing the eosinophil counts obtained in the different cases before, during and after treatment, the number of eosinophils per c.mm. found at each examination is expressed in Table III in terms of the normal eosinophil count for India which on the basis of the work by ACTON and DHARSIENDRA (1933) and NAPIER and DAS GUPTA (1935) is taken as 500 per c.mm.

The eosinophil records obtained from the commencement to the end of the 1st week after completion of treatment varied greatly in relation to the original counts prior to treatment. In four cases (Cases 20 1 b and 13) the eosinophilia increased (by 2,000 to 21 600 per c.mm.) although in one of these cases (Case 13) the count subsequently fell to less than the original by the end of the 1st week. In the remaining six cases for which records during this period were available a definite but variable reduction in eosinophilia occurred, the counts ranging from 1,300 to 9,810 per c.mm. as compared with initial counts

of 7,560 to 18,450 per c.mm. for this group. During the 2nd week after completion of treatment (six records) the reductions in eosinophilia were considerable and the number of eosinophils less than 5 000 per c mm. except in one case (Case 9) where the count still exceeded 20 000 per c.mm. During the 3rd and 4th weeks after treatment the records (seven) suggest that reduction in eosinophilia was continuing and that in some cases (Cases 12, 3 and 15) it was approximating to normal. Subsequent records from the 2nd to 7th months after completion of treatment show still further reductions and indicate that these were general and progressive. Of the final eosinophil counts (sixteen) made during this period nine were less than 1 000 per c mm. five were between 1,000 and 1 600 per c.mm. and two exceeded 2,000 per c.mm. the range in these cases being from 280 to 4 480 per c mm. as compared with 8,920 to 42,550 per c.mm. prior to treatment.

### SPUTUM FINDINGS

Although considerable prominence was given in our previous paper to the importance of protecting the samples of sputum from contamination by mites from extraneous sources further experience has served to emphasize that the precautionary measures adopted must be of a high standard of efficiency and carried out with scrupulous attention to detail. Accordingly in order to reduce the possibilities of such contamination to an absolute minimum, certain modifications of the technique originally described were introduced early in these investigations. Screw-capped bottles were discarded and flasks or large tubes with tight fitting rubber stoppers only were used. These were prepared as previously described and were then placed in cylinders containing a solution of pyrethrum (about 1 inch in depth) the covers of which were sealed with vaseline impregnated with the same insecticide. Directions issued with the apparatus emphasized the importance of retaining the flask or tube within the cylinder at all times except during expectoration. The flasks and tubes when issued each contained 10 c.c. of 1 per cent. potassium hydroxide this acted as a preservative and also reduced the period required for disintegration of the muco-purulent material in the laboratory. On arrival at the laboratory the sputum samples were, if necessary treated with additional potassium hydroxide and formalin and were then replaced in the cylinders or kept in a refrigerator until such time as they could be examined. All reagents used in the work were filtered and kept in the refrigerator. In every case the whole sample (usually a 24-hour sample) of sputum was centrifuged and the deposit examined under a low power binocular microscope.

In the course of these investigations over 200 samples of sputum were examined, and mites of various species were found at one time or another in the sputum of every patient except Case 21 who was unfortunately unable to produce any material for examination. Mites were also found in the urine



of three of the cases from whom samples were submitted owing to concomitant urinary disorders.

In Table IV the results of the examinations of sputum from each patient before during and after treatment, are given. Those for the last category are meagre owing mainly to the fact that, by the time treatment was completed, the production of sputum was much reduced and several patients were unable to provide specimens for examination.

On reference to the table it will be seen that whereas mites were found in the sputum of twenty three of the twenty four cases examined before treatment, they were present only in fifteen of twenty three cases during the period of treatment and that they were found in forty three (50.6 per cent.) of eighty five samples of sputum examined before treatment and in thirty-seven (36.6 per cent.) of 101 samples taken during treatment. But the actual number of mites (ninety five) recovered from the mite positive samples (forty-three) before treatment was definitely less than the number (122) recovered from the mite-positive samples (thirty seven) during treatment. In the majority of instances the number of mites present in any one positive sample of sputum collected prior to treatment was low rarely more than one or two, and exceeding three on six occasions only and in one instance alone was a considerable number of mites found in a single sample, *viz.* fifteen mites in the fifth sample collected from Case 9 who at the time was suffering from an acute attack of asthma. During treatment, the number of mites present in the positive samples was also usually low but it exceeded three on eleven occasions and was considerable (ten and over) in four samples. In three cases (Cases 3, 7 and 10) mites were numerous in the samples collected on the first 3 days of treatment in Case 3 thirty mites were found in the first two samples, in Case 7 eleven mites were present in a portion of the sample (the rest having been lost by an accident) collected on the 2nd day of treatment, and in Case 10, twenty-four mites were present in the first three samples that collected on the 3rd day containing fifteen. In the sputum samples (twenty four) collected shortly after completion of treatment, mites were less often present and less numerous than in the samples taken either before or during treatment, and in no instance was more than a single mite found in any one of the positive samples. But further observations in respect of this last category are necessary.

#### *TYPES OF MITES PRESENT IN THE SPUTUM*

The mites found in the sputum of the cases studied were, for the most part of types similar to those mentioned in our previous paper (1944), and were essentially those which are commonly associated with stored products, dust and debris. They included a wide range of species, several of which it has not yet been possible to identify. The majority (approximately 75 per cent.), however belonged to the families Tarsonemidae and Tyroglyphidae. The Tarsonemid mites were of two kinds, the small species of *Tarsonemus*

previously noted and a species of *Pediculoides* or allied genus. The former occurred in the sputum of nineteen of the patients and in 40 per cent. of the mite-positive samples in all cases except Case 6—in whom a single mite only was found—it was associated with other species. In Cases 7 and 10 it was the predominant mite present, the numbers of *Tarsonemus* found in each being fourteen and seventeen respectively. Males appeared to be more prevalent than previously observed, and were found in 14 per cent. of the positive samples, larvae were isolated on three occasions and a single egg—containing a fully formed larva—was found in the sputum of Case 7. In this case twelve specimens (five females five males one larva and an egg) were present in the sputum samples collected on the 2nd and 3rd days after commencement of treatment. The species of *Pediculoides* (?) was found in small numbers in six of the patients most of the specimens isolated were females, but one larva was found. This mite is a very small (approximately  $150\mu$  in length) delicate species which may be easily overlooked when examining sputum all specimens seen appeared to be of the same species which, however was not *P. ventricosus* the mite commonly associated with grain and responsible for the so-called "grain itch" in persons handling infested materials. The Tyroglyphid mites found included several species of *Tyroglyphus* and *Glyciphagus* and one of an undetermined genus which agrees with KRAMER'S (1899) definition of *Mealia* in that the anus and anal suckers of the male are surrounded by a chitinous annular sclerite. Mites of this group occurred in the sputum of 17 cases and in 53.6 per cent. of the mite-positive samples. Species (at least two) of *Tyroglyphus* were the most prevalent and were found in all stages from egg to adult in the sputum of fifty five specimens isolated, nineteen were adults, six were nymphs, five hypopial nymphs four larvae, five eggs and the remainder nymphs or adults in too severely macerated a condition to allow of accurate determination. In Case 3 the samples of sputum taken during the first 2 days of treatment yielded thirty mites in various stages of these sixteen were *Tyroglyphus*. The occurrence of hypopial nymphs in the sputum is a matter of considerable interest. Such nymphs differ greatly in appearance from the normal nymphs, each being provided with a hard convex dorsal shield and a ventral disc armed with powerful suckers. Some authors regard this stage as an interpolatory one produced mainly for purposes of migration the factors responsible for its formation are not clearly understood, but in this stage the mite is known to be highly resistant to unfavourable conditions.

Of the remaining mites found in the sputum in this investigation little can be said until their identification is known. Among them, however were nine specimens (from five cases) of Parasitid mites and five specimens (from four cases) of Cheyletids. Single specimens of *Demodex Eriophyes* (?) *Hoploderma* and of the human itch mite, *Sarcoptes scabiei* were also isolated. The last named was in the larval stage but was badly macerated.

The mites found in the urine of Cases 1, 2 and 11 were mainly *Tyroglyphus*, *Tarsonemids* and an unidentified species. Early stages of *Tyroglyphus longior* (?)—an egg and a larva—and of the unidentified mite—larva and nymphs—were found in addition to adults of both sexes.

#### SOME CONSIDERATIONS ON THE POSSIBLE RELATION OF MITES TO CERTAIN BRONCHIAL DISORDERS.

The results of our earlier investigations on the presence of mites in human sputum led us to believe that the evidence obtained suggested (a) that the mites found in the sputum were derived from the lungs and/or bronchi (b) that in one case at least they were probably living and breeding in the respiratory tract (c) that the chief method of infection was by inhalation (d) the infestation with mites was in some cases of long duration and (e) that the condition variously known as pseudo-tuberculosis, eosinophil lung and tropical eosinophilia might, in part at least, be explained on the basis of mite infestation of the respiratory system.

The present investigations have not elicited any facts necessitating modification of these views rather the additional evidence obtained tends to support them.

In our opinion the strict precautionary technique adopted in these investigations in respect of the collection and examination of sputum samples has entirely precluded the possibility of contamination of the samples by mites from extraneous sources. This being so it can only be concluded that the mites were derived from the patients and since examinations of mouth and nasal washings have so far proved negative and mites have only been found in sputum, it would seem that they were derived from the lower part of the respiratory tract. Furthermore the types of mites recovered from the sputum were essentially those which normally occur among materials—stored goods, foodstuffs, litter and dust and debris of various kinds—with which man is frequently associated in varying degrees of intimacy dependent mainly upon the nature of his environment. The influence of the latter factor is directly reflected in the present series of cases by the fact that at least eight of them were engaged in occupations which involved close association with potential mite-bearing materials and possibly also by WEINGARTEN's (1943) statement that of seventeen cases of tropical eosinophilia seen by him in Bilmer, sixteen belonged to the merchant communities of Calcutta and Bombay. In tropical countries particularly however it would be unreasonable to regard the possibility of mite infestation as limited to persons who work in stores, warehouses, factories, mills and so forth, for conditions favouring multiplication of mites of the types specified are often present in and around the dwellings of the poorer residents. In this connection STORM VAN LEEUWEN (1923), in discussing "climate asthma" in Holland, states that 90 per cent. of the allergic cases are hypersensitive to allergens in the air which are absorbed by the

mucous membranes of the air passages, he considered that the most important factors in this group were 'climatic allergens (or miasmas) which consist of products of destruction of micro-organisms mould and insects (among the last mites being important) which occur for the most part inside our houses.

Inhalation is believed to be the chief method by which the mites gain entrance to the air passages but no precise information is yet available on the extent to which mites become airborne following disturbances—due to wind cleaning processes, or removal and rearrangement of materials—of their breeding places and immediate surroundings. It is however reasonable to think that the prevalence of airborne mites will vary greatly and accordingly that wide differences in the degree of infestation are likely to occur among infected persons. The occasional inhalation of a single living mite may well be a matter of common occurrence, and may have little or no effect upon the recipient except in rare instances. But should circumstances ensure more frequent inhalation of mites the chances of some of the mites establishing temporary residence in the respiratory tract would probably be increased and even although the period of residence be limited, the continued accessions of freshly inhaled mites might well result in a series of overlapping infestations involving the continued presence of living mites over long periods. HINMAN and KAMPMEIER (1943) considered that Tyroglyphid mites were unable to establish themselves for protracted periods in the alimentary tract and that it was the long continued ingestion of food infested with these mites which caused the severe diarrhoea in their experimental dogs. Nevertheless the possibility that certain species of Tyroglyphid and Tarsonemid mites may become established in the respiratory system and live and even breed therein over relatively long periods deserves consideration. Indeed, we suggest that this possibility is by no means remote. The powers of adaptation and resistance to unusual conditions exhibited by these mites are considerable and the expectoration of relatively large numbers of particular species of mites in all stages by patients shortly after administration of arsenic has now been observed in three cases (one in the previous investigations) and would seem to indicate that breeding was in progress. But evidence that Tyroglyphid mites are able to live in human tissue is also afforded by the finding on two occasions of living mites in accretions of fresh sputum and by the presence of all stages from eggs to adults in urine and also by the remarkable cases recorded by TROUËSSART (quoted by HINMAN and KAMPMEIER) and HOPE SIMPSON (1944). In the former case a scrotal cyst on puncture yielded some 2 ounces of fluid containing approximately 800 mites of the Tyroglyphid genus *Histiogaster* in all stages of development in the latter a carcinoma of the jaw was found to be infested by *Tyroglyphus* (possibly *T. longior*) and living mites eggs and faecal matter were present in the growth suggesting that the mites had been resident for a considerable period in the situation in which they were found. In this connection too it may be apposite to observe that at some time in their evolution the free living ancestors of the parasitic lung

mites of monkeys (*Pneumonyssus*) and some other animals passed through a phase during which adaptation to the conditions existing in the respiratory system occurred.

The number of mites found in the sputum of the cases investigated varied considerably both before and during treatment. Often few or single specimens only were seen in large samples of sputum, but occasionally numerous mites occurred in a single sample. The prevalence of mites in the sputum of an infected person however would not necessarily bear direct relation to the intensity of the infestation. Both the condition of the mites present and their site of residence would probably influence the actual numbers expectorated. Dead mites would conceivably be more readily expectorated than living ones and mites living in the larger bronchioles would be more easily expelled than those in the smaller bronchioles or alveoli, particularly if the former were occluded by pus or mucus, or the latter were collapsed. Moreover the presence of large numbers of mites may not always be necessary to produce the symptoms observed for VELLANBY (1943) has shown that the number of parasites actually present in human scabies is usually small and much less than would be expected from the extensive signs and symptoms associated with clinical scabies.

The possible effects upon the individual of living mites in the respiratory tract may next be considered. *Pneumonyssus* is definitely pathogenic: it invades the lungs of monkeys and leads a parasitic existence causing extensive cavitation and a condition simulating tuberculosis. There is no evidence at present, however, to suggest that non parasitic mites of the types which have so far been found in the respiratory system of man are capable of producing similar lesions. But several authors consider that the existence of Tyroglyphid mites in the stomach and intestines may produce both in man and animals pathological symptoms of an allergic type. That this is a feasible proposition is apparent from the fact that their mere external contact with man frequently causes severe dermatitis thus affording evidence that these mites are capable of producing powerful toxins (DOWLING and THOMAS, 1942).

Present opinion tends to the view that the function of the eosinophil is to neutralize toxins of protein origin (BLACKLOCK and SOUTHWELL, 1931) and that an excess of eosinophils in the blood indicates the presence of some foreign animal protein or of certain amines which can be derived from the amino-acids of plants or animals (ACTON and DHARASENDRA, 1933). The latter authors also showed that reactivity to protein varied in different individuals, and that the degree of eosinophilic response depended on the sensitivity of the individual to particular proteins. STROVO (1942) considered eosinophilia to be an allergic response as it occurred after repeated injections of a foreign protein and after a latent period. Since in several of our cases a high initial eosinophilia obtained when no animal parasites other than mites could be demonstrated, there is good reason to believe that the latter were responsible for this condition. The presence of mites in the respiratory tract, however would not necessarily be associated with an excessive eosinophilia. A person in whom the

infestation is in an early stage might show only a slight eosinophilia, and a non-sensitive, or non sensitized individual might show no definite reaction. MELLANBY (1943) has recently demonstrated that in scabies infections in man sensitization usually occurs about one month after the initial infection and that during the period preceding sensitization no discomfort is caused by the activities of the itch mites but on subsequent reinfection an intense local irritation occurs within a few hours of the entry of the mite into the skin.

The relation of eosinophilia to allergic manifestations has been indicated by CODE (quoted by STRONG 1942) who reported that the eosinophil is a carrier of histamine and that the existence of this substance in such cells may partly explain the mechanism present in asthma, hay fever and other allergic conditions. Histamine, a cleavage product of protein, may be produced in the body by the breaking down of an introduced protein or of body tissue and it is therefore probable that it, or a closely allied substance, may be liberated by the disintegration of dead organisms, by toxic emanations from living organisms or by damage done by them to the tissues. Thus the mechanism involved in the production of those forms of asthma which are associated with a high eosinophilia would seem to be, first, increased activity of the eosinophils due to the presence of a foreign protein, second, the liberation of histamine from the protein by one or more of the means indicated above, and subsequently the absorption of the histamine by the eosinophils and its transportation by them to the vaso-motor centres where the asthmatic spasm is initiated.

That asthma may result from the disintegration of dead insects or portions of insects in the respiratory tract is shown by FIGLEY'S (1929) observation in Toledo where cases occurred among persons who had inhaled the pelts and fragments of May flies and who had become sensitized by long exposure for successive seasons to the annual invasions of myriads of these insects from Lake Erie. HANSEN (1929), EARLE (1944) and others have described cases of asthma due to adult *Ascaris* and their emanations, in whom the asthma persisted as long as the patients were infested with the worms but disappeared directly the latter were expelled by treatment with specific drugs.

DILLING (1943) considers that neoarsphenamine after circulating in the blood as a colloid becomes adsorbed both on the parasites and tissue cells which slowly oxidize it into a toxic trivalent compound. The arsenic is stored in the spleen, liver, lungs and kidneys and excretion occurs mainly into the faeces and urine. An observation made on one of our cases to whom 1.25 grammes of carbarsone (28.85 per cent. arsenic) were administered over a period of 57 hours showed that 0.008 mg. of arsenious oxide (0.006 mg. arsenic) were excreted in 53 c.c. of sputum expectorated by the patient during that time. We have also found that arsenic, in the form of arsenious oxide (1:1000) kills *Tyroglyphus longior* in 10 minutes and in the form of neoarsphenamine (1:1,000) in 24 hours.

The striking and rapid effects of organic arsenicals—the great reduction

in eosinophilia and cessation of clinical symptoms—upon those cases in the series in whom no parasites other than mites were found, suggests, therefore, that these effects resulted from the elimination of living mites by the parasitical action of the drugs. The possibility of some unrecognized parasite, such as spirochaetes which are also susceptible to arsenic being present in our cases and being the causative agents of the condition has been considered but so far has not received support from experimental work which will be described in a subsequent paper.

### SUMMARY

1 An account is given of investigations conducted on twenty-five Ceylonese patients, all of whom showed high eosinophilia (not less than 3,000 per c mm.) and, with one exception, symptoms of respiratory disorders—chiefly bronchial asthma.

2 Case histories are summarized in tabular form, but six of special interest are given in detail. The duration of the symptoms at the time the patients came under investigation varied from 3 weeks to 17 years.

3 All cases were treated with organic arsenicals and with one exception, responded satisfactorily. The response was rapid and involved a great reduction in the eosinophilia and cessation of the clinical symptoms. These effects were maintained throughout observation periods of from 4 to 11 months.

4 The records available indicate that during and for the first 2 weeks after completion of treatment the eosinophilia varied. In some cases it increased, but in the majority it decreased considerably. From the 3rd week after treatment onwards the reduction in eosinophilia became general and pronounced.

5 Over 200 samples of sputum were examined and mites of various species were found at one time or another in every patient from whom samples were obtained. Mites were also found in the urine of three of the cases with concomitant urinary disorders. Usually the number of mites present in a sputum sample was small but in five samples collected from four cases it was relatively high, ranging from ten to twenty in each sample. Mites were present in 50.6 per cent. of the samples collected before treatment, in 36.6 per cent. of those examined during treatment and in 25 per cent. of those examined after treatment, but the average number of mites found in each positive sample in the last category was definitely lower than in the other categories.

6 The types of mites found in the sputum were essentially those which are commonly associated with stored products, dust and debris. Approximately 75 per cent. belonged to the families Tarsonemidae and Tyroglyphidae. All stages of mites—including hypopial nymphs of a species of Tyroglyphid—were found in the sputum and in two patients all stages of the same species occurred in two successive 24-hour samples of their sputum.

7 The relation of mites to certain bronchial disorders is discussed with reference to the results of these and the previous investigations.

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TABLE I  
PARTICULARS OF CASES INVESTIGATED

Case No.	Sex and Age	Occupation	Duration of asthma	Clinical diagnosis	Radiographic findings	Examination of sputa (S) and urine (U).	Examination of sputum for TB.	Treatment.	
								Date started.	Total amount given in grains
1	F 10	School girl	8 years	Chronic bronchitis and emphysema	June 1912. Appearances suggestive of chronic bronchitis	7.0.12 (U) B (S) Normal. 7.10.12 13. (U) Seven samples examined—mucus (total 25) present in all but last sample 14.12.12. (S) Tracheitis	Two samples, both negative	14.12.12 8.1.14	1.43 0.65
2	F 45	—	8 years	Bronchial asthma and cystitis	—	15.1.12. (U) Normal. 16.1.12. 15. (U) Normal. 6 percent. 19.12.12 and 12.1.14 (U) Normal	—	12.1.14	2.6
2	M., 23	Petty salesman in bakery	8 months	Bronchial asthma	"8.12.12. "Mottling of both lungs—Lymphatic congestion." 27.1.14 "Appearances those of peribronchial fibrosis—most of areas of mottling disappeared." 25.7.14. "Lungs clear—no evidence of disease."	8.1.14 (S) "111 miliary ova, scanty"	Four samples, all negative	3.1.14 9.1.14	3.18 2.08
4	M., 35	Assistant manager in bank	Intermittent asthma over 10 years	Asthmatic bronchitis	27.1.14 "No evidence of actual disease of lungs; appearances attributable to miliary tuberculosis"	—	Several samples, all negative	29.1.14	0.6

6.	M., 23	Quartermaster	3 weeks	Bronchial asthma	10.1.44 "Marked reaction of floor lung markings with pleural thickenings on right side. No active infiltration seen"	10.2.44 (U) Normal (S) Normal (U) Normal (S) Few red blood corpuscles and pus cells	Five samples all negative	4.3.44	3.9
6.	M. 26	Trader	About 1 year	Bronchial asthma	28.2.44 Marked peribronchial fibrosis with reticulation. 12.3.44 "No definite evidence of disease of lung"	10.2.44 (S) Normal	10.2.44, Negative	4.3.44	3.9
7	M. 56	Trader	6 years	Chronic bronchial asthma	17.2.44 Appearance of diffuse fibrosis of lungs	—	Five samples all negative	26.2.44	3.9
8	M. 16	Garden labourer	About 1 year	Emphysema with infiltration of lung	27.11.43 "Suspicious mottling with peribronchial fibrosis over bases" 28.2.44 "Peribronchial fibrosis with reticulation (? chronic asthmatic bronchitis)"	26.2.44 (S) "Well mottled, scanty"	Three samples (one culture) all negative	7.3.44	3.0
9	M. 20	Student	3 months	Bronchial asthma	—	—	Dec., 1913 Negative	3.3.44 26.5.44	2.6 1.56
10	M., 20	Student and subsequently Naval rating	Intermittent over 7 years	Bronchial asthma	19.2.44. All areas of both lungs show marked increase of tissue fibrosis emphysema and gross and fine mottling. An ill-defined area at 3rd right interspace suggestive of cavitation. Appearance suggestive of asthma but TB cannot be excluded	25.2.44 - 11.3.44 Four samples (S) examined. Ova of <i>Trichuris</i> , <i>A. stercaria</i> and <i>Ancylostoma</i> present. Samples of 10 and 11.3.44 negative	Three samples all negative	12.3.44	5.2
11	P. 55	—	4 years	Bronchial asthma and pyelitis	—	16.3.44 (U) One mite present in sample 20.3.44 (U) Normal	7.3.44 Negative	19.3.44	2.0

TABLE 1—continued.

Case No.	Sex and age	Occupation	Duration of illness	Clinical diagnosis	Radiographic findings	Examination of sputum for TB.	Treatment	
							Date started	Total amount given in grams.
12	M, 24	Conductor on ocean steamer	Several years	Bronchial asthma	March, 1911. F. adenocarcinoma bronchus with emphysema noted in lung field. No active infiltration. Appearances seem to be expected for an aged thymatic attack.	19341 Negative	17444	8.01
12	M., 27	Steward	3 months	Bronchial asthma	Feb. 1911. No evidence of disease. Increase of asthma typical of asthma.	1-3.44 and 1.2.44. (9) Ora of Trachea and Aortic bronchus. 10 and 1-4.44 Normal	21244	5.5
11	M., 39	Tailor	4 years	Bronchial asthma	1910. "No evidence of disease."	1940 Negative	19244 19.2.44	2.4 1.06
15	F, 40	---	5 years	Bronchial asthma	---	1912 Negative	22.4.44	2.4
16	M., 31	Naval rating	6 months	Bronchial asthma	25.4.44. "Cardiac shadow normal, some increase of hilar shadow with little peribronchial thickening in right lower zone. Right costal hump. No pericardial involvement."	23.4.44. (U) Normal. 19.4.44 (B) Normal	3.8.44	2.26
17	M., 47	Miner	Many days	Acute bronchitis and asthma	---	23.4.44. (U) Normal. 19.4.44 (B) Normal	9.8.44	2.4

18	M, 25	Engineer	Since child hood	Bronchial asthma	23.5.44. "Appearances suggestive of bronchial asthma"	—	Feb. 1941 Negative	0.6.44 17.6.44	2.50 3.0
19	M., 25	Clerk	6 months	Bronchial asthma	27.5.44 Mottling of right apex suggestive of early disease	May 1941 (S) Normal	27.5.44 Negative	0.6.45	0.6
20	F., 40	—	0 years	Bronchial asthma	Feb. 1944 "No evidence of disease"	—	Feb. 1944 Negative	17.6.44 0.8.44	2.6 2.0
21	M., 40	Lab. assistant	—	No symptoms of disease other than high eosinophilia. Past history of chronic bronchitis from 1939 to 1941	—	Several samples of stools and urine examined all normal	Several samples examined all normal	17.7.44	2.08
22	M. 20	Carpenter	4 years	Bronchial asthma	—	—	—	15.7.44 2.8.44	2.75 1.5
23	M. 40	Record clerk	4 years	Chronic catarrhal bronchitis	1940 suggestive of chronic bronchitis	Several samples of stools examined—all normal	1940 Negative	*8.7.44	4.0
24	M., 6	Stores clerk	1 year	Bronchial asthma	—	—	Nov 1943 Negative	3.8.44	4.16
25	M., 31	Stores clerk	17 years	Bronchial asthma	—	—	—	0.8.44 2.8.44	3.0 1.56

TABLE 11

BLOOD FINDINGS (MEANS PER CASE)

Case No.	Before treatment.				After treatment with arsenic.				
	Date of examination.	Neutrophils	Lymphocytes	Eosinophils (per cent.)	Date of examination.	No. of days from completion of treatment.	Neutrophils	Lymphocytes	Eosinophils (per cent.)
1	8.13.43	7,400	4,800	70-2	5.11 1.4.41	65 (48) 108 (81)	3,940 4,070	5,640 4,620	3.84 1.10
2	9.12.43	---	---	68.4	18.4.41 17.6.41	91 151	---	---	---
3	9.12.43	6,710	5,300	50-0	11.1.44 18.1.44 4.4.44 9.7.44	3 11 27 (13%) 203 (180%)	5,750 4,000 3,870 5,300	2,440 1,900 2,420 4,600	10.20 4.70 7.90 1.10
4	12.1.44	5,500	5,000	77-0	9.2.44 23.2.44 9.5.44	13 7 11	4,100 3,250 7,500	3,000 3,400 4,070	1.00 1.70 9.70
5	21.1.44	2,010	6,500	74.8	15.4.44	72	4,420	1,400	50
6	11.2.44	2,800	2,200	61-0	1.11.45	---	2,320	2,500	1.40
7	17.2.44	7,820	6,000	47-0	2.2.44	1	5,800	2,120	21-0
8	26.1.44	2,220	8,420	63-0	14.2.44	3	2,010	2,400	81-0
9	17.2.44 28.2.44	4,770 5,760	4,400 4,270	73-0 72-0	10.2.44 20.4.44 15.6.44 20.8.44	8 43 64 (10%) 270 (180%)	4,270 3,870 2,040 0.300	6,400 2,890 2,810 2,000	81.00 4.02 2.03 1.30
10	18.2.44	7,900	2,000	41.8	27.8.44	84	2,700	2,000	28.0

12.	10.3 44	5,710	5,250	8,020	40-0	31.3 41 15.5 41	3 47	7 450 4 100	5 750 5 150	5 550 1 000	35-0 17 0
13.	10.3 44	5 820	5,250	8,020	40-0	31.3 41 15.5 41	3 47	7 450 4 100	5 750 5 150	5 550 1 000	35-0 17 0
14.	18.3 44	4 070	5,540	30 640	07-0	12.5 41 12.5 41 2.10 44	27 41 184 (130*)	4 800 6 000 5 250	8,480 3 050 4 370	3,320 1 150 780	30 0 6 0 7 5
15.	17 4 44	5,050	4,450	3 000	23 4	23.5 41	*4	5 630	4,650	520	8 2
16.	26 4 44	3 640	3,380	7 500	54-0	17.5 41	8	3,550	3 150	1 300	18-0
17.	16.5 44	—	—	—	68 1	31.7 41	46	2 450	3 600	1 050	21 5
18.	25 5 44	3,050	6,050	14 000	59 2	10.7 41 31.8 44 20.10 44	17 69 (7*) 121 (02*)	5 600 5 100 3 500	6,900 6,200 4 800	2,800 3,000 500	18 5 20 5 0 8
19.	27 5 44	3,160	5 160	9 500	58-0	21 0 44	0	0 050	4 100	3 450	25 4
20.	10 0 44	3 800	6,850	42 550	80-0	14.7 44 14 10 44	21 115 (58*)	— 3 100	— 3 070	— 4 480	65-0 40-0
21.	10 7 44	3,750	5 100	25 150	74-0	*5.8 44 17 10 44	30 80	2,850 1 470	3 780 3 950	1 800 880	21-4 0 6
22.	11 7 44	11 350	8 050	9 400	33 5	22.7 41 20.9 44	2 68 (40*)	4 800 2,570	4 000 4 150	6 300 680	30 5 0 2
23.	12.7 44	2,700	3 200	18 100	75 4	25.8 41 10 10 44	18 55	3 400 4 400	2,850 2,050	2,150 1 150	25 6 14-0
24.	27 7 44	2,080	3,670	3 050	33 8	24.8 44 17 11 44	13 98	2,500 1 400	4 450 5 130	900 1 070	11 3 10 1
25.	3 8 44	8,300	5 050	18 400	57 5	10.8 41 28.8 44 10 10 44	1 10 52 (30*)	0 000 2 960 4 140	5 070 4 070 5 930	0 810 2,070 0 90	44-0 29 7 8 5

Days from completion of second course of treatment.

This patient was in hospital previously in July 1943 for cough and asthma on 30th July a blood count gave total leucocytes 77 000 with eosinophils 70.840 (92 per cent.).

TABLE III

REDUCTION OF EOSINOPHILS SUBSEQUENT TO TREATMENT WITH ARSENIC.  
(Normal Eosinophil count—500 per c.mm.—as a quotient of actual count found.)

Case No.	Eosino- phils prior to treat- ment.	Eosino- phils during treat- ment.	Eosinophils after completion of treatment.						
			1st month.			2nd month.	3rd month.	4th month.	5th & 7th months.
			1st week.	2nd week.	3rd and 4th weeks.				
40	85.1	—	104.7	—	—	—	—	8.9	—
4	73.2	—	—	5.2	3.5	—	—	1.9	—
1	57.6	61.2	—	—	—	—	4.6	4.3	—
9	53.1	—	—	45.6	—	8.0	—	4.7 and 4.9	8
5.	60.9	—	—	—	—	—	0.6	—	—
1.	50.3	—	—	—	—	3.4	1.1	—	—
11	43.3	—	—	—	—	—	—	—	1.4
14	41.3	—	—	—	0.6	3*	—	—	1.4
10.	36.9	18.4	5.0	—	—	0.6	—	—	—
5.	36.6	—	19.6	5.9*	—	1.8	—	—	—
23.	36.2	—	—	—	4.3	* 3	—	—	—
1.	35.5	—	—	—	0.5	—	—	—	—
8	34.8	—	57.1	—	—	—	—	—	—
18.	28.0	—	—	—	5.6	—	5.8	—	1.4
7	24.8	—	18.8	—	—	—	—	—	—
3.	4.0	—	18.8	9.4	1.5	—	—	—	2
6	19.6	—	—	—	—	—	—	—	2.8
22.	18.8	—	1.6	—	—*	—	1.3	—	—
19.	18.6	—	—	6.9	—	—	—	—	—
13.	1.9	49.6 and 61.1	13.1	—	—	3	—	—	—
16.	15.1	13	6	—	—	—	—	—	—
4.	6.1	—	—	1.8	—	—	—	2.1	—
15.	6.0	—	—	—	1.8	—	—	—	—

In Cases 40, 1, 9, 14, 23, 18, 3 and 22 second courses of treatment were given at intervals of from 9 days to 11 weeks after completion of the first treatments; the approximate time at which the second treatment occurred is shown in the table by an asterisk.

TABLE IV  
EXAMINATION OF SPUTUM FOR MITES.

Case No.	Before treatment.			During treatment.			After treatment.		
	No of samples examined.	No with mites.	Total No of mites found.	No of samples examined	No with mites.	Total No of mites found	No of samples examined.	No with mites.	Total No of mites found.
1.	6	3	4	8	3	3	1	Nil	Nil
2.	3	2	3	3	Nil	Nil	—	—	—
3.	3	2	7	6	3	31	3	1	1
4.	2	1	3	5	3	8	—	—	—
5.	3	1	2	3	Nil	Nil	—	—	—
6.	4	1	1	3	Nil	Nil	—	—	—
7.	3	1	2	4	4	15*	4	Nil	Nil
8.	5	1	1	3	Nil	Nil	2	Nil	Nil
9.	5	3	20	5	3	4	2	Nil	Nil
10.	•	1	1	7	3	24	—	—	—
11.	2	2	2	5	4	12	—	—	—
12.	3	3	8	—	—	—	—	—	—
13.	5	5	14	6	3	4	1	1	1
14.	2	2	6	3	2	5	1	1	1
15.	3	1	1	5	3	4	—	—	—
16.	5	3	3	5	Nil	Nil	—	—	—
17.	1	1	1	1	1	1	—	—	—
18.	9	3	3	4	Nil	Nil	1	Nil	Nil
19.	•	1	5	2	Nil	Nil	3	1	1
20.	3	1	1	6	1	1	—	—	—
22.	3	1	2	3	Nil	Nil	1	1	1
23.	1	Nil	Nil	3	1	4	1	Nil	Nil
4.	5	2	2	6	1	1	2	1	1
25.	5	2	3	5	2	5	2	Nil	Nil
—	85	43	95	101	37	122	24	6	6

\* Part of the sample collected on the second day of treatment was lost by accident.





STUDIES ON THE VENOM OF SOUTH AFRICAN SCORPIONS  
(*PARABUTHUS*, *HADOGENES OPISTHOPHTHALMUS*) AND THE  
PREPARATION OF A SPECIFIC ANTI SCORPION SERUM

BY

E. GRASSET M.D.

A SCHAAFSMA B.Sc.

AND

J. A. HODGSON B.Sc.\*

*The Serum Department South African Institute for Medical Research,  
Johannesburg*

These investigations were initiated in 1940 in response to a request from the Union Defence authorities for the preparation of an anti scorpion serum owing to the presence of large numbers of scorpions in certain districts where troops were encamped. This measure was of a precautionary nature as very few severe cases of scorpion sting had been reported in the Army. Although various species of scorpions are widely distributed throughout South Africa

\* We wish to acknowledge our thanks to the following persons for their valuable assistance in these investigations: Dr I B POLK EVANS (Irene) Mr BLACKBEARD (Serowe) and the TRANSVAAL PONGOLA DEVELOPMENT COMPANY for collecting thousands of scorpions. Dr R. F. LAWRENCE, Director of the Natal Museum, Pietermaritzburg, who classified the scorpions sent to him. Dr J. H. MASON, Research Veterinary Officer of the Serum Department. Mr J. CHALMERS, Veterinary Officer of the Serum Department. Miss K. M. WOOLDRIDGE and Mr F. A. BRANDT for their invaluable technical assistance and finally to our colleagues Dr D. A. LOUW (Brandvlei) Dr G. HOLTZHAUSEN (Pofadder) Dr A. T. B. H. BODENSTADT (Groblersdal) Dr D. MALHERRE (Philippstown) Dr B. BOYER (Johannesburg) Drs. J. VAN EDEN and J. O. MALLEY IRWIN (Thabazimbi) for supplying us with clinical data on the effects of anti-scorpion serum.

and scorpion stings are not infrequent especially among the Native population, fatal cases are exceptional and are observed usually in children.

No statistical data on this subject could be obtained either from the Union Public Health Department or the Director General of Medical Services in the Union. Furthermore the almost complete lack of scientific data on the relative toxicity of the various South African scorpions apart from a study on *Opisthophthalmus capensis* (EASDIN 1933) made it necessary to undertake a study of the venom of the main representatives of South African scorpions. In contrast, comprehensive studies have been made of the zoological taxonomy and systematics of South African scorpions. All our zoological references have been taken from HEWITT'S (1925) important monograph on South African scorpions and the more recent contribution of LAWRENCE (1942) which refers chiefly to Natal and Zululand scorpions.

The investigations detailed in the present paper are limited to the following three genera (1) *Parabuthus* (2) *Hadogenes* (3) *Opisthophthalmus*.

These genera are the most common representatives of the South African scorpion fauna and constitute by far the vast majority of the specimens sent to this Institute by various collectors. A certain number of *Uroplectes* (Butidae) and *Cheloctonus* and *Opisthocanthus* (Scorpionidae sub-family Iachnurae) were received but in too small numbers to be of experimental value.

#### GEOGRAPHICAL DISTRIBUTION

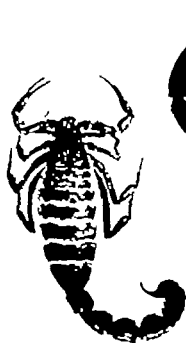
*Parabuthus* (Family Butidae).—This occurs throughout Africa and Arabia but is apparently absent from Natal and Pondoland. Our specimens were sent from the National Pongola Reserve Northern Transvaal and Bechuanaland Protectorate. Most of the specimens were of the two following species *P. transvaalicus* and *P. bibrachius*. Average weight of adult specimen = 4.5 grammes, average length from anterior margin of carapace to tip of telson = 7.2 cm. *Parabuthus* belongs to the same family as *Buthus* *peringuestratus* commonly found throughout the whole of Northern Africa and responsible for most of the severe stings reported in Egypt. (TOOD 1909). These two genera present similar zoological characteristics. In contrast to the *Opisthophthalmus* and *Hadogenes* which are armed with powerful pincers (pedipalps) but have a slender tail and elongated telson, the Butidae have thin pincers but possess a massive tail ending in a powerful chitinous telson. (See Photograph 1.)

*Opisthophthalmus* (Family Scorpionidae sub-family Scorpioninae).—This occurs abundantly throughout South Africa, extending northwards to the Camero river in the West and equatorial regions in the East. Most of our specimens came from the Bechuanaland Protectorate and two species were received, *O. schultzei* and *O. phyllorhynchus*. The average weight of adult specimens received was 6.7 grammes, the average length = 8.3 cm.

*Hadogenes*. (Family Scorpionidae sub-family Iachnurae).—This occurs in South Africa and Madagascar North as far as the Feira district, Portuguese East Africa, West to Congo territory. It is, apparently absent from the coastal strip from Capetown to Port Elizabeth. The majority of specimens received belonged to the same species *Hadogenes tragolytes dentatus* and were sent to us from the Eastern Transvaal. Average weight of adult specimen = 6 grammes, average length = 10.2 cm. (The maximum weight recorded was 18.45 grammes for a *Hadogenes* female 18 cm. in length.)

#### EXTRACTION OF VENOM

The method of extracting scorpion venom usually adopted by various workers on a large scale is based on mechanical crushing of dry telsons followed by aqueous maceration.



*Parabuthus*  
Average length 7.2 cm.

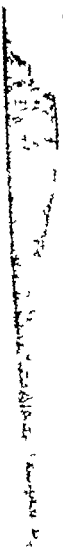
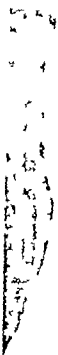
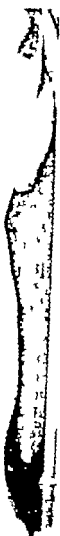


*Hadogenes*  
Average length 10.2 cm.



*Opisthophthalmus*  
Average length 8.2 cm.

PHOTOGRAPH 1 — Specimens of South African Scorpions dealt with in this paper



According to TODD (1909) telsons are dried in the sun and then placed in a desiccator over calcium chloride. When the venom was required the dry telsons were ground in a Turkish coffee mill and added to 0.8 per cent saline in the proportion of one sting to 1 ml. saline.

SERGEANT (1938) at the Pasteur Institute Algiers dried the cut telsons at 37° C. which he kept thereafter at room temperature in sealed tubes until they were required. For experimental work the telsons were ground up, macerated in physiological saline and extraction was completed with glass beads. SHULOV (1939) in Palestine MAHOMED (1942) in Egypt and MAGALHAES (1939) in Brazil used similar methods.

C. PHISALIX and M. PHISALIX (1922) obtained the venom by applying an electric current to the telsons of the scorpions. While this method undoubtedly was an improved technique for individual specimens, SERGEANT who tried the Phisalix method on 397 *Proxenus australis* states that the small yield of venom (an average of 1.3 mg.) so obtained does not advocate its use for large-scale collection of venom. A similar method was used by ELDON (1933) in South Africa on *Opisthophthalmus capensis*. Our method of extracting scorpion venom differed from the above techniques. We endeavoured to collect scorpion venom on a large scale in as pure a form as possible.

For *Parabuthus* possessing hard chitinous telsons the best method was to extract venom from the telson by aspiration with a fine capillary pipette, after the tip of the telson had been cut. The thin layer of liquid venom collected on a watch glass was left at room temperature exposed to air and dried within a few hours. For *Hadogenes* and *Opisthophthalmus* the chitinous telsons of which are easily collapsible the method was to squeeze the telson glands between the arms of a pair of forceps and collect the liquid venom on a watch glass. After desiccation, the potency of the pooled venom was estimated in relation to its dry weight in milligrammes as in the case of snake venoms. The only reference we have found of a similar technique is that mentioned by KRAUSS (KOLLE, KRAUSS and UHLENBUTH, 1930) working on South American scorpions (*Tityus*).

### *Physical properties of scorpion venom*

After extraction, the liquid venom had the appearance of a white milky viscous fluid. No appreciable difference was observed between the venoms of the three genera. The pH of the venoms was determined electrometrically with a Beckman pH meter the values were pH 6.4 for *Parabuthus* venom, pH 5.5 for *Opisthophthalmus* venom and pH 6.2 for *Hadogenes* venom. After desiccation the venom took the form of a white flaky product adhering to the watch glass from which it could be detached with a needle in the form of fine scales.

The weight of the venom was determined by the difference between the weight of the watch glass before collection and after desiccation of the venom. The weight of the liquid venom immediately after collection was also taken, to record the percentage loss of moisture on drying. The total number of scorpions used for each collection was noted usually 50 to 100 for each watch glass which allowed for the determination of the average figure of dry venom per scorpion collected.

1. *Parabuthus*—Venom was collected from 338 specimens. The total weight of venom collected was 1.6342 grammes. The average yield was 4.8 mg. of dry venom per scorpion. Insufficient scorpions arrived at any one time to allow weight of liquid venom being taken with accuracy.

2. *Opisthophthalmus*—For a period of 3½ years venom was collected from 5,985 specimens. The total weight of venom collected was 8.6225

grammes. The average amount of wet venom per scorpion was 6.5 mg. the average yield of dry venom was 1.4 mg. giving a 78.5 per cent. loss of weight on drying. This figure compares closely with that given by EADIN (1939) for the average dry weight of venom obtained per scorpion from *O. capensis*, 1.3 mg.

3 *Hadogenes*.—During the same period venom was also collected from 9603 specimens of this genus. The total weight of venom collected was 26.2791 grammes. The average amount of wet venom per scorpion was 12 mg. the average yield of dry venom was 2.7 mg., giving a 77.3 per cent. loss of weight on drying.

No significant seasonal difference was detectable either in the appearance or the weight of the venoms collected. The total number of scorpions used for all experiments was 16,946.

#### SYMPTOMS FOLLOWING INJECTION OF SCORPION VENOM TO LABORATORY ANIMALS AND THE DETERMINATION OF THE MINIMUM LETHAL DOSE.

For experimental work, a given amount of saline was added to dry scorpion venom to obtain a solution containing 10 mg. per c.c. Solution was facilitated by mechanical stirring with a glass capillary pipette. The solution was left to stand to allow debris to settle. The supernatant venom solution was then used without filtration or centrifugation. In order to minimize possible individual differences pooled venom samples have been used in each experiment.

Experimental intoxication was studied on mice, guinea-pigs, rabbits and pigeons injected by various routes: intravenous, subcutaneous, intramuscular, intracutaneous and intracerebral. Fresh venom solutions were used in all experiments it being found that the venom was unstable in solution. All experiments were completed on the day in which venom was put into solution.

#### *Parabuthus* VENOM.

*Parabuthus* venom. *White mice* (23 to 22 grammes).—The intravenous injection of 0.1 to 0.4 mg. of *Parabuthus* venom (about 1/40 to 1/10 telson) into white mice in a volume of 0.5 c.c. was followed immediately by acceleration of respiration, dyspnoea, staggering and inco-ordination of movement—hind legs stretched in forced extension with tail flexed over back—in other cases mice sat erect, gasped, jumped about uncontrollably and fell paralysed. Death occurred after signs of asphyxiation from 30 seconds to 5 minutes after injection. In some cases death was delayed up to 30 minutes after progressive paralysis and dyspnoea. Injection of sublethal doses resulted in the same type of symptoms, followed by gradual recovery.

Subcutaneous injections of 0.5 to 2.0 mg. of *Parabuthus* venom are necessary to produce death. In spite of the high dosage onset of the symptoms is often delayed up to 30 minutes, death occurring in 2 to 3 hours after progressive dyspnoea and paralysis.

Intracerebral injections were carried out with a venom concentration of 40 mg venom per c.c. to minimize the volume of inoculum to 0.05 c.c. The venom injected varied from 0.0001 mg to 0.2 mg. Doses up to 0.0005 mg did not affect mice and produced only mild transitory symptoms. Injection of doses from 0.002 to 0.1 mg were followed by immediate convulsions, trembling, paralysis with the hind legs stretched out. Death occurred from within a few seconds to 3 hours according to dosage. Injection of 0.1 mg to 0.2 mg resulted in almost immediate death.

*Parabuthus venom Guineapigs (350 to 400 grammes)*—Guineapigs were injected subcutaneously and intramuscularly. The lethal dose of *Parabuthus* venom was 1 to 2 mg. Immediately on injection the animals showed signs of acute discomfort and pain by squeaking, biting the area of injection, coughing and gasping for air. There was distension of abdomen. All had a white exudate around the eyes and symptoms of extreme salivation before death, which takes place in 1 to 3 hours. In some cases the heart continued beating for several minutes after failure of respiration. There were no haemorrhagic lesions.

Guineapigs were also injected intracutaneously to ascertain haemorrhagic action of venom. Thirty minutes after injection of 0.5 to 1.0 mg erection of hairs is observed, followed by symptoms described above. At the site of the injection there were signs of vaso-constriction with a 10 mm. area of blanching. Death occurs in  $1\frac{1}{2}$  to 4 hours after progressive prostration and asphyxiation.

*Parabuthus venom Rabbits (2,000 grammes)*—A rabbit injected intracutaneously in three places in the depilated skin of the abdomen with 4 mg, 2 mg and 1 mg of *Parabuthus* venom gave similar symptoms. The 4 mg injection resulted in a very slight haemorrhagic infiltration of 20 mm. diameter which enlarged to 30 mm. after 5 hours. The 2 mg injection gave a slight haemorrhagic infiltration, while the 1 mg injection showed no visible alteration in the tissue. The rabbit developed paralysis in the hindquarters after 5 hours and succumbed in 6 hours with signs of progressive asphyxiation.

The intravenous injection of 5 mg in a volume of 4 c.c. was followed by rapidly increasing signs of dyspnoea and gasping. The rabbit was most sensitive to touch, cringing away. This was followed by trembling and uncontrolled rolling on the floor. Death ensued in 5 to 10 minutes with signs of asphyxiation. At autopsy immediately after failure of respiration the heart was found to be still beating. There were no apparent lesions of the internal organs nor haemorrhage of the muscles.

*Parabuthus venom Pigeons (400 to 450 grammes)*—The intravenous injection of 0.8 to 1.5 mg of *Parabuthus* venom in a volume of 2 c.c. resulted in the appearance of immediate symptoms of intoxication, the birds lying on their chests or side with legs extended under the tail, gasping, sneezing, choking and with the head arched over the back. During the following minutes these neuromotor symptoms were aggravated, respiration became gradually



more difficult and the bird died with signs of asphyxiation from 5 to 20 minutes after injection.

The intravenous injection of sublethal doses, i.e. 0.5 to 0.7 mg. of venom, was followed by immediate paresis, inability to fly and choking. After a few hours these symptoms gradually lessened and the bird recovered. The injection of doses up to 0.4 mg. did not result in apparent disorders.

The minimum lethal doses of *Parabuthus* venom are given in Table I.

TABLE I

Animals.	Intravenous Injection.	Intramuscular Injection.	Subcutaneous Injection.	Intracerebral Injection.
Mice	0.1-0.4 mg. (2-10 min.)	0.5- 0 mg. (1 hr.)	0.5-2.4 mg. (1-3 hr.)	0.001-0.002 mg. (3-120 min.)
Guinea-pigs	—	1-0.4 mg. (1-2 hr.)	1-0.4 mg. (2-3 hr.)	—
Rabbits	6 mg. (8 min.)	—	—	—
Pigeons	0.5-1.5 mg. (3-20 min.)	1-2 mg. (5-10 hr.)	—	—

From these experiments it is seen that the symptomology following injections of *Parabuthus* venom into animals by various routes, is essentially the same. *Parabuthus* venom contains a neurotoxic substance which exerts a specific action on the neuromotor and respiratory centres. After a period of neuromuscular hyper-excitability paralysis ensues; death is due to asphyxiation by paralysis of the respiratory muscles. This action is very rapid when venom is introduced intracerebrally or intravenously—delayed and progressive when injections are made subcutaneously or intracutaneously. The toxic action of *Parabuthus* appears to be similar to that of *Buthus occitanus* and *B. garrae* (PHILALIX, 1922).

### *Opisthophthalmus* VENOM

*Opisthophthalmus* venom. Mice.—The intravenous injection of mice in doses of 2 to 3 mg. (1 to 2 telsona) of *Opisthophthalmus* venom resulted in similar symptoms described above: increased rhythm of respiration, gasping soon followed by progressive paralysis and death in 1 to 20 minutes.

Subcutaneous injections from 5 to 17 mg. were followed in 2½ hours by oedematous swelling at the site of injection, which in the higher doses spread to the abdomen with evidence of pain squeaking and irritability. Death ensued in 24 to 36 hours.

Intracerebral injections of 0.0002 mg. to 0.002 mg. gave no apparent

symptoms. Injections of 0.005 to 0.007 mg. resulted in immediate convulsions but did not cause death. With injections of 0.01 to 0.02 mg. the mice rolled from side to side, with convulsive movements, death following from 1 to 5 minutes after injection.

*Opisthophthalmus venom Guineapigs*—Guineapigs injected subcutaneously with doses from 20 to 80 mg. of venom showed a haemorrhagic oedema at the site of injection, after 1 hour the intensity of the reaction was proportional to the dose. After 16 to 24 hours the subcutaneous tissue of the abdomen was distended by a gelatinous haemorrhagic fluid. This area, which may be as large as 40 mm. in diameter became necrotic and sloughing occurred followed later by the formation of a hard deep scab with well delimited edges. In no case was involvement of the peritoneal cavity observed. Notwithstanding the severity of the lesions the animals recovered.

Guineapigs were injected intramuscularly in the internal portion of the thigh with doses of 20 to 80 mg. in a volume of 0.5 to 2 c.c. Within 10 minutes after injection of 20 mg. there was a retraction of the leg injected, which was maintained in semi flexion. During the following 30 minutes the limb became swollen, showing tense haemorrhagic infiltration extending to the subcutaneous tissue. After 2 to 3 hours the whole leg was distended from the groin to the extremity with purple-violet ecchymosis and complete loss of all active movement of the limb. In spite of the severity of the local lesions the animals remained in relatively good condition with no apparent dyspnoea or paralysis of the limbs not injected. After 24 hours haemorrhagic fluid oozed from several places in the distended limb. This was soon followed by sloughing of the whole necrotic mass of muscles and skin, leaving the bone exposed. The animals eventually recovered after loss of the leg by spontaneous amputation.

With the injection of higher doses of 40 to 80 mg. the haemorrhagic swelling involved the flank and abdomen with extensive oozing. After sloughing of the leg tissues, the limb remained hanging in a disarticulated position. This was followed in 36 hours by spontaneous amputation accompanied by a very offensive smell. The whole picture resembled experimental intoxication by gas-gangrene toxins i.e. *B. perfringens* and *B. histolyticus* toxins. Death ensued within 36 to 48 hours.

At autopsy the whole of the abdominal wall and adjacent thoracic portion were infiltrated with a haemorrhagic gelatinous oedema—this was more intense in the groin and in the remaining necrotic upper part of the limb. The peritoneal cavity was not involved. There were no apparent lesions in the internal organs nor any generalized haemorrhagic syndrome.

Intracutaneous injection in the guineapig resulted in a haemorrhagic patch of tegument appearing immediately at the site of injection, followed by a lesion differentiated into two zones—a whitish avascularized centre surrounded by a haemorrhagic ring (Photograph 2). *Opisthophthalmus* venom differs mainly from that of *Hadogenes* in its higher activity. With a low dose

of 2 to 12 mg of *Opisthophthalmus* venom the cutaneous lesion is about twice the diameter of the lesion formed when *Hadogenes* venom is employed. With a higher dose the same results hold and, in addition necrosis starts earlier than it does when *Hadogenes* venom of the same dosage is used. The lesion, however remains localized. After a large scab has formed, healing ensues leaving a wide, thickened scar with permanent loss of hair.

*Opisthophthalmus* venom. *Rabbit*—Rabbits given an intravenous injection of 10 mg of *Opisthophthalmus* venom showed signs of discomfort after 5 minutes, but no further signs were observed. Other rabbits when injected with 15 and 20 mg showed signs of staggering after 1 minute. This was followed immediately after by uncontrolled jumping and rolling and the hind-legs were paralysed in forced extension. The animals died with signs of asphyxia in 2 minutes.

*Opisthophthalmus* venom. *Pigeons*—Pigeons were submitted to intramuscular injections of *Opisthophthalmus* venom in the pectoral muscle with doses from 10 to 50 mg in a volume of 1 c.c. A dose of 10 mg did not produce any apparent symptoms except local haemorrhage. The injection of 20 to 50 mg was followed 2 to 3 hours later by inco-ordination of movement, staggering and inability to stand or fly. Birds lay on their chests with legs and claws in full extension under the tail, and wings flapping ineffectually. With higher doses such as 50 mg death occurred in 3 hours. Occasionally delayed death was observed with the injection of 20 to 30 mg. On sectioning apart from a haemorrhagic area in the pectoral muscles, no internal lesions were found.

Pigeons injected subcutaneously with doses of 10 to 60 mg. in the pectoral region showed within 2 to 3 hours a haemorrhagic infiltration at the site of injection with some swelling. Although no actual paralysis was observed as in the case of intramuscular injection, the same inability to fly was shown and birds remained standing. Death was observed with the highest doses. Similar symptoms, using the venom of *O. capensis* have been described by Eason (1933).

The minimum lethal doses of *Opisthophthalmus* venom are given in table II.

#### *Hadogenes* VENOM.

*Hadogenes* venom. *Mice*—Mice injected intravenously with doses of 0.2 to 0.3 mg (1/10 telson) did not show apparent symptoms. With doses of 0.4 to 0.6 mg. (1/5 to 1/3 telson) dyspnoea was observed after a few minutes followed by signs of paralysis, with the hind legs in forced extension. Death occurred within 1 to 15 minutes. At autopsy no lesions were to be seen. The clotting of the blood was normal.

Mice injected subcutaneously with the surprisingly large lethal doses of 30 to 40 mg showed, during the minutes following injection, an increased rhythm of respiration accompanied by irritability running about, erratic

TABLE II

Animals.	Intravenous Injection	Intramuscular Injection	Subcutaneous Injection.	Intracerebral Injection
Mice	1-3 mg (3-20 min.)	10-15 mg (*4-36 hr.)	10-15 mg (24-36 hr.)	0.01 mg (2-10 min.)
Guineapigs	—	60 mg (48 hr.)	>80 mg (104 hr.)	—
Rabbits	15-20 mg (2 min.)	—	—	—
Pigeons	3 mg (6 min.)	50 mg (3 hr.)	50 mg (3½ hr.)	—

jumping and biting of each other, with obvious severe pain at the site of injection. This first period of 1 to 2 hours of hyper-excitability was followed by trembling and progressive prostration the animal lying on its abdomen or side with increasing dyspnoea. Death occurred 3 to 40 hours after injection.

Intracerebral injections of 0.002 to 0.02 mg of venom were necessary to produce signs of intoxication such as staggering a hunching up of the body and general paresis. After a period of 24 hours immobility the animal gradually recovered. With doses of 0.05 to 0.1 mg the following symptoms appeared almost immediately: animal turned from side to side or lay on its back with extremities stretched out and head in forced contraction forwards on the chest. It remained immobilized with occasional spells of inco-ordinate movement. Death took place in from 5 minutes to 2 hours. Finally with doses of 0.1 mg death followed within 4 to 8 minutes.

*Hadogenes venom. Guinea-pigs*—The subcutaneous injection of guinea pigs with the still more surprising lethal doses of 100 to 185 mg gave much the same picture as that seen in the subcutaneous injection of mice. Here, however at the site of injection a large saccular oedema containing about 10 c.c. of haemorrhagic fluid was formed. In a surviving pig this gradually dried and became covered with a hard necrosis. Death occurred in about 22 hours.

The intramuscular injection of 80 mg of venom into the leg of a guinea pig only caused swelling and redness the pig remaining alive.

Detailed experiments on the intracutaneous action of *Hadogenes* venom in guinea-pigs have been done with doses covering the very wide range of 2 to 20 mg. White, depilated pigs of 600 grammes were injected with four decreasing doses in the flanks (a maximum of two on each side to avoid overlapping). The injection of 2 mg was sufficient to produce within the following minutes a haemorrhagic patch of 5 mm which was still evident after 24 hours. Injections of 4 mg., 8 mg and 12 mg resulted in haemorrhagic reactions of 10 mm

15 mm. and 20 mm. respectively. On touching them, there were signs of pain. These haemorrhagic lesions do not disappear on prolonged pressure of the finger proving the permanent nature of the lesion. They are followed by necrosis and scab formation. With doses of 16 to 20 mg. the haemorrhagic patch of 25 mm. which rapidly followed after the injection of the venom, soon differentiated into two zones—a whitish central avascularized zone of 20 mm. diameter surrounded by an external violet haemorrhagic ring of 2 to 3 mm. After 24 hours the central white area became necrotic and sloughed off in a mass leaving the normal subcutaneous tissue exposed. Regeneration took place from the periphery of the wound by granulation. (See Photograph 2.) During the whole evolution of the lesions the animals remained in good condition. The only general symptom observed soon after the injection apart from obvious pain was one of nervous excitability.

*Hadogenes venom. Rabbits*—The intravenous injection of 25 mg. of *Hadogenes* venom in 8 c.c. of saline into rabbits did not produce any apparent symptoms of intoxication. The injection of 50 mg. resulted in death within 15 minutes. There were no visible lesions at autopsy and the clotting of the blood was normal.

A freshly depleted rabbit was injected intracutaneously with 4 mg., 2 mg. and 1 mg. At the site of the 4 mg. injection there appeared within the following minutes an area of marked congestion and hypervascularization of 40 mm. diameter without haemorrhagic phenomena. After reaching its maximum during the following 30 minutes it gradually faded away and had practically disappeared in 5 hours. The 2 mg. and 1 mg. injections did not produce any appreciable skin reaction.

*Hadogenes venom. Pigeons*—Pigeons were submitted to intravenous injections of *Hadogenes* venom in doses of 2 mg. to 10 mg. in a volume of 2 c.c. of saline. The average M.L.D. was found to be 8 mg. With sublethal doses of 2 to 4 mg. the main symptom was the extension of the legs backwards immediately after the injection, accompanied by the inability of the bird to fly. These early symptoms disappeared after a minute or two—the bird was able to stand and recovered soon after. With lethal doses of 6 to 10 mg. the same signs appeared while the injection was still taking place. The birds remained lying on their chests or sides with shaking of the head which was sometimes flexed backwards, gasping, trembling of the whole body and violent unco-ordinated movements of the wings and body. This first phase was followed by progressive prostration, death supervening in 2 to 10 minutes. At autopsy there were no external haemorrhages, visible lesions of the organs, or any abnormality in blood-clotting.

Pigeons were injected intramuscularly with 40 mg., 60 mg. and 80 mg. of venom, respectively. Only with the highest doses were symptoms noted. Here the leg injected was retracted and swollen and there was a serous exudate

at the site of injection. All the birds survived. It was necessary to inject 125 mg to cause death which then took place after 10 minutes.

The minimum lethal doses of *Hadogenes* venom are given in Table III.

TABLE III

Animals.	Intravenous Injection.	Intramuscular Injection	Subcutaneous Injection	Intracerebral Injection
Mice	0.2-0.6 mg (3-15 min.)	30 mg (2.0 min.)	30-40 mg (6-36 hr.)	0.05 mg. (0.90 min.)
Guinea-pigs	—	>80 mg	125 mg (22 hr.)	—
Rabbits	50 mg (15 min.)	—	—	—
Pigeons	6-8 mg (6-10 min.)	125 mg (10 min.)	125 mg. (4-12 hr.)	—

### HAEMOLYTIC ACTION OF SCORPION VENOMS ON ERYTHROCYTES *in vitro*

#### TESTS WITH SHEEP ERYTHROCYTES.

In a first experiment, decreasing amounts of venom from 20 mg to 1/100 mg per c.c. in a volume of 1 c.c. were added to a 2 per cent suspension of washed sheep erythrocytes. The final concentration of sheep red cells was thus 1 per cent. The mixtures were placed in the incubator at 37° C for 1 hour after which they were read with the naked eye.

For *Parabuthus* venom slight haemolysis (about 3 per cent.) was observed in the tube containing only the highest dose of venom. For *Opisthophthalmus* venom 5 to 8 per cent. haemolysis was observed—again only in the tube containing 20 mg venom. No haemolysis was present in any mixture containing *Hadogenes* venom. In a second test with 0.1 per cent. concentration of sheep cells, 20 mg of *Parabuthus* venom had no haemolytic effect, while the same amount of *Opisthophthalmus* venom caused complete (+++) haemolysis. The same large dose of *Hadogenes* venom caused only a trace of haemolysis.

#### COMPARATIVE TESTS WITH SHEEP AND GUINEA-PIG ERYTHROCYTES

In the following comparative experiments with sheep and guinea-pig red cells, venom solutions over a range of 40 mg to 1 mg per c.c. were used. Readings were taken after 1 hour incubation at 37° C. Owing to the insufficient quantity of *Parabuthus* venom available comparative tests with this venom could not be undertaken; however experiments performed with it demonstrated only a slight haemolytic action.

TABLE IV  
*Hadogenes venom.*

Amount of Venom.	1 Sheep Cell Suspension.	0.2 Sheep Cell Suspension.	1/2 Guineapig Cell Suspension.	0.2 Guineapig Cell Suspension.
40 mg.	+	+	+	±
20 mg.	±	++	++	+
10 mg.	±	0	++	++
1 mg.	0	0	+++	+++
control	0	0	0	0

TABLE V  
*Opisthophthalmus venom.*

Amount of Venom.	1 Sheep Cell Suspension.	0.2 Sheep Cell Suspension.	1/2 Guineapig Cell Suspension.	0.2 Guineapig Cell Suspension.
40 mg.	++	±	++	+
20 mg.	++	±	++	+
10 mg.	+	++	+++	++
1 mg.	±	+	+++	++
control	0	0	0	0

The experiment was repeated with guineapig red cells, using venom solutions in dilutions of 20 mg. to 0.01 mg. per c.c. to determine the end point of haemolytic action.

### Conclusions

From these experiments it appears that a haemolytic action is exerted by the three venoms under test only when used in relatively high concentrations. All conditions being equal the tests show the greater sensitivity of guineapig erythrocytes to haemolysis, as compared with sheep red cells. It is also more marked on 0.2 per cent. cell suspensions than in the case of the 1 per cent. suspensions.

These findings confirm those of EADEN (1933) working on *Opisthophthalmus capensis* who also found that guineapig cells were the most sensitive. A similar haemolytic action has been observed by KUBOTA (1918) with the venom of the Manchurian scorpion *Buthus martensii* and the venom of the *Vicras Centruus exilicanda* (1918a). In contrast no haemolytic action could be

TABLE VI  
*Hadogenes* AND *Opisthophthalmus* VENOM

Amount of Venom.	<i>Hadogenes</i> Venom.		<i>Opisthophthalmus</i> Venom.	
	1 Guinea-pig Cell Suspension.	0.2 " Guinea-pig Cell Suspension.	1 Guinea-pig Cell Suspension.	0.2 " Guinea-pig Cell Suspension.
20 mg.	++	+	++	+
10 mg.	++	++	+++	++
5 mg.	+++	++	+++	++
1 mg.	+++	+++	+++	+++
0.5 mg.	±	+++	0	±
0.1 mg.	0	0	0	0
0.01 mg.	0	0	0	0
control	0	0	0	0

observed by HOUSSEY (1919) on the venom of the African *Buthus quinquestratus* (as quoted by M. PHILALIX 1922) even after the addition of lecithin. Similar negative results have been reported by TODD (1909) with pooled Egyptian scorpion venom (apparently *Buthus* and *Prionurus*).

#### EFFECT OF HEAT ON *Opisthophthalmus* AND *Hadogenes* VENOM *Opisthophthalmus* venom

An experiment was carried out to ascertain the effect of heat on a sample of venom. Fractions of a 10 mg per c.c. solution of the venom were heated in a water bath to (a) 70° C for 30 minutes (b) boiling point, which is 93° C at 6 000 feet altitude, for 30 minutes (c) boiling point for 90 minutes.

Mice were injected intravenously with 0.3 mg of each of these fractions (This was the average M.L.D. of this batch of venom, the mice dying 1 to 4 minutes after injection.) Heating the venom to 70° C delayed the average time of death to 8 minutes after injection. Heating the venom to boiling point for 30 minutes delayed death for 10 minutes and heating the venom at boiling point for 90 minutes delayed the average time of death for 13 minutes after injection.

Intracutaneous injections of 5 mg of these venom fractions into guinea-pigs produced oedematous, haemorrhagic local reactions of 20 mm. diameter which reached their maximum intensity in 48 hours. These later altered to dry necrotic scabs. There was no difference in the intensity or in the final effect seen between the unheated and heated fractions of the venom.



A similar experiment was carried out on another sample of venom. Fractions of a 40 mg per c.c. solution of the venom were heated to 50° C., 60° C. and 70° C. for 15 minutes each, another fraction to 80° C. for 30 minutes. An unheated fraction was used as a control. Of each of these fractions, three doses, corresponding to 4 mg., 12 mg. and 20 mg. of venom, were injected intracutaneously into depilated guinea-pigs. The injection of 4 mg. of unheated venom was followed 4 hours later by a haemorrhagic patch 10 mm. in diameter. The haemorrhagic areas resulting from the injection of 12 mg. and 20 mg. of venom, were respectively 30 mm. and 40 mm. in diameter. Although these injections were done on the same animal at a distance of 4 to 6 cm. apart, an extensive oedema enveloping the whole of the abdomen was caused. Injections of similar doses of the fractions of venom heated to 50° C., 60° C., 70° C. and 80° C. produced reactions of 10 mm., 20 mm. and 20 mm. diameter. Notwithstanding the comparatively smaller reactions, they were still definitely of a haemorrhagic nature. No appreciable difference in the intensity of the reactions could be observed in the four heated fractions. The oedema caused by the injections of the heated venoms was also comparable though this was less than that caused by the unheated venom.

These experiments tend to show the limited destructive action of heat on the toxic and haemorrhagic principles of *Opisthophthalmus* venom. EXNER (1933) found that the venom of *O. capensis* loses its toxicity on prolonged heating to 100° C. but does not say to what extent this occurs.

#### *Hadogenes* venom.

Experiments to ascertain the effect of heat on *Hadogenes* venom were also carried out. Fractions of a 10 mg per c.c. solution of venom were heated to (a) 70° C. for 30 minutes (b) boiling point for 30 minutes and (c) boiling point for 90 minutes. Amounts of 0.2 mg., 0.3 mg., 0.5 mg. and 1 mg. were injected intravenously into mice. Similar amounts of unheated venom were injected into mice to act as controls. The results are set out in Table VII.

Guinea-pigs were injected intracutaneously with 5 mg. of each of the unheated and heated fractions of venom. An early oedematous reaction, followed by a haemorrhagic and necrotic lesion, was caused at the site of injection. This was of an equal degree of intensity in all four pigs. Another sample of venom was dissolved in saline to obtain a 40 mg per c.c. solution. In this case fractions of the solution were heated to 50° C., 70° C. and boiling point for 1 hour respectively. Three doses, corresponding to 4 mg., 12 mg. and 20 mg. were injected intracutaneously into depilated guinea-pigs. Similar amounts of unheated venom were used as controls. The results are set out in Table VIII.

Forty-eight hours after the injection the haemorrhagic reactions described above were transformed in all cases into necrotic lesions. These all healed later by granulation from the sides. The pigs were not affected. These

TABLE VII

Amount of <i>Hadogenes</i> Venom.	Unheated Venom.	Venom heated to 70° C. for 30 min	Venom heated to 93° C. (B.P.) for 30 min	Venom heated to 93° C. (B.P.) for 90 min.
0.3 mg.	Survived	Survived	Survived	Survived
0.5 mg.	Survived	Survived	Survived	Survived
	Died—6 min	Survived	Died—8 min	Survived
	Died—18 min	Survived	Died—13 min	Died—6 min
0.7 mg.	Died—3 min.	Died—2 min	Died—2 min.	Died—8 min
	Died—3 min.	Died—15 min	Died—5 min.	Died—15 min
1.0 mg.	Died—1 min	Died—1 min	Died—5 min.	Died—1 min
	Died—1 min.	Died—8 min.	Died—5 min	Died—3 min.

TABLE VIII

<i>Hadogenes</i> Venom.	Amount of Venom Injected	Hæmorrhagic Reaction	Intensity of Reaction
Unheated venom	mg	mm.	
	4	5	++
	12	10	+++
	20	15	++++
Heated to 90° C—1 hr	4	5	+
	12	10	++
	20	15	+++
Heated to 70° C—1 hr	4	2.5	+
	12	7	++
	20	10	+++
Heated to 93° C—1 hr	4	2.5	+
	12	5	+±
	20	7.5	++

experiments again show the limited action of heat on the toxic and hæmorrhagic principles of *Hadogenes* venom.

#### PROTEOLYTIC ACTION OF SCORPION VENOMS ON GELATIN *in vitro*

To determine the proteolytic action of scorpion venom we used the same technique as in our studies on snake venoms (GRASSET and SCHAAFSMA, 1940). To a series of tubes containing 0.3 c.c. of 20 per cent. gelatin in physiological

saline (kept in water bath at 40° C.) decreasing amounts of a 40 mg per c.c. venom solution were added, i.e., 30 mg to 0.1 mg. The volume in all tubes was brought up to 1 c.c. by the addition of saline. The final concentration of gelatin was, therefore 6 per cent. which was found sufficient for the control tubes to solidify at 6° C. after 30 minutes. After addition of the venom solutions the mixtures were kept at 37° C. for 5 hours and then placed in the refrigerator at 6° C. to allow the gelatin to solidify. Readings of the tubes were taken after 30 minutes and again after 12 hours. In all cases comprehensive pooled mixtures of venom were used. The results obtained are given in Table IX.

TABLE IX.

Amount of Venom	<i>Parabuthus</i> Venom	<i>Opisophthalmus</i> Venom	<i>Heteros</i> Venom
30 mg	Liquefaction +++	No liquefaction	No liquefaction
7 mg	Liquefaction ++	No softening	No softening
10 mg	Liquefaction +	(	0
3 mg	Softening	0	0
mg	(	0	0
1 mg	(	0	0
0.3 mg	"	0	0
0.1 mg.	(	0	0
control	0	0	0

Repeated experiments carried out with other mixtures of venoms led to similar results. Thus only *Parabuthus* venom was found to exert a definite proteolytic action on gelatin and that only when used in relatively high concentrations. This is in contrast to the findings of HOUSSAY (1919), quoted by PINHALEX (1922) who found that the glandular extracts of *Buthus* spp. have no enzymic action on gelatin, starch and casein.

### IMMUNOLOGICAL INVESTIGATIONS

#### NEUTRALIZATION OF SCORPION VENOM BY POLYVALENT

##### *Naja flava* *Bilis arietans* ANTIVENENE.

With a view to ascertaining the possible neutralization of South African scorpion venom by snake antivenene tests were carried out with the polyvalent antivenene prepared at this Institute. One c.c. of this concentrated antivenene neutralizes either 1 mg. of the venom of *N. flava* or 10 mg. of the venom of *B. arietans*.

In a first experiment, 0.1 mg. (1 M.L.D.) of a pooled mixture of *Parabuthus* venom was mixed with 0.4 c.c. of antivenene. After 1 hour contact at 37° C.

white mice of 20 grammes were injected intravenously with the mixtures. Five out of the six mice injected died within 6 to 20 minutes. Of six control mice injected with 0.1 mg (1 M.L.D.) of venom alone four died within 5 minutes.

Experiments carried out with *Opisthophthalmus* and *Hadogenes* venom gave similar results. No protection thus appeared to be exerted by this polyvalent antivenene.

Anticobra serum was also found to have no protective action against the venoms of *Heterometrus maurus* (NICOLE and CATOUIILLARD 1905) *Buthus quinquestratus* or *Tityus bahiensis* (HOUSSAY 1919). The same absence of group neutralization has been observed when using antilachesis and anticrotalic serum.

Although clinical data of persons stung by South African scorpions and treated by injection of our polyvalent *N. flava* *B. anelans* antivenene tended to show that the pain symptoms were minimized, there were no active proofs of venom neutralization. In view of these facts it was decided to undertake the preparation of a specific anti-scorpion serum from the venom of the scorpion species available.

#### PREPARATION OF SPECIFIC ANTI SCORPION SERUM

Anti-scorpion sera have been prepared in several countries where the incidence and gravity of scorpion stings warranted the preparation of a specific antidote. In Egypt, an anti-scorpion serum active against *Buthus quinquestratus* and *Prionurus* was prepared by TODD (1909) and recently perfected by MOHAMMED (1942). A similar type of therapeutic serum against the venom of *B. quinquestratus* has also been manufactured by the Lester Institute (from dry telsons sent from Egypt to England) and also by SHULOV (1939) in Palestine. In Algiers, SERGENT (1936) prepared an anti-scorpion serum against the venom of *Prionurus australis*, the most toxic species in Northern Africa. This anti-serum is also active against the venoms of *Prionurus lionvillieri* and *Buthus occitans*. In Brazil, the preparation of a polyvalent anti-scorpion serum has been evolved by VITAL BRAZIL at the Butantan Institute and at the Ezequiel Dias Institute by MAGALHAES against species of South American scorpions (*Tityus bahiensis* and *T. serrulatus*).

#### DETOXICATION OF SCORPION VENOM.

Considering the satisfactory results obtained in the preparation of various snake antivenenes by the use of formalized venoms or anavenoms (GRASSET & ZOUTENDYK, 1932 and 33) attempts were made to apply similar methods to the preparation of anti-scorpion serum. As in the case of snake venoms the scorpion venom concentration used for detoxication was 10 mg per c.c. The yield of venom from 378 scorpions of the genus *Opisthophthalmus* i.e., 673 mg., was dissolved in 67.3 c.c. of saline. After sedimentation of the organic debris the solution was slightly centrifuged. The supernatant fluid had a white turbid appearance and the pH which was determined electrometrically was 5.52. Determination of the toxicity of a sample of this venom solution showed a M.L.D. of 3 mg by the intravenous route for mice. (Death in 5 to 20 minutes.)

This 10 mg. per c.c. solution was split into two fractions—one had 0.5 per cent. and the other 0.8 per cent. of 40 per cent. formol added. The two fractions were incubated at 37° C. After a period of 15 days incubation the two formolized venoms were tested. As preliminary tests by means of intravenous injections given to mice tended to show that the residual formol was lethal for these animals, the two products were dialyzed for 24 hours. The intravenous injection of 5 to 8 mg. of the dialyzed, detoxicated venoms, representing two to three lethal doses of the original venom, was not followed by any apparent symptoms of toxicity. A similar method of detoxication was used for *Hadogenes* and *Parabuthus* venoms. The resulting detoxicated antigens were used for the immunization of a horse.

*Immunization of a horse with detoxicated and unaltered scorpion venom.*

Horse 394 was submitted to the following series of subcutaneous injections of detoxicated scorpion venoms at 3- to 5-day intervals. The first doses of 10 mg. and 20 mg. of formolized *Opisthophthalmus* venom were progressively increased to 100 mg. Then *Hadogenes* detoxicated venom was substituted in doses of 20 mg. to 200 mg. The immunization was then continued with *Parabuthus* venom. At the end of 3 months the horse had received an amount of 2,210 mg. of detoxicated scorpion venom comprising respectively 1,235 mg. *Hadogenes*, 195 mg. *Opisthophthalmus* and 680 mg. *Parabuthus* venom. The immunization was completed with injections of unaltered *Hadogenes* venom up to a total of 872 mg. spread over four injections. The total amount of scorpion venom injected during the course of immunization from 8.3.41 to 29.5.41 was just under 3 grammes. No undue reactions were observed subsequent to the injection of detoxicated and unaltered venoms. Owing to the lack of venom, the immunization could not be continued. The horse was bled 9 days after the last injection.

*Neutralization tests with immunized horse serum.*

Considering the fact that immunization was to the greater part carried out with *Hadogenes* venom the assays on the activity of serum dealt mainly with that venom. Mice and pigeons were used as experimental animals. In all these assays, scorpion venom solutions were used within the hours following preparation, as it has been our experience that, for *Hadogenes* venom, the toxicity of the original venom drops very quickly after dissolution. For each experiment the lethal dose by intravenous injection into pigeons weighing 350 grammes was determined before the neutralization tests were started. One c.c. of serum from Horse 394 was then mixed with increasing multiples of 1 to 4 M.L.D. of *Hadogenes* venom for intravenous injection into the pigeon. Details of the results of the tests are given in the following table—

One c.c. of anti-scorpion serum therefore protected against 24 mg. venom (three lethal doses for the pigeon). Similar neutralization tests with the immune serum from Horse 394 carried out with ten pooled samples of *Hadogenes* venom on pigeons, gave analogous results. 1 c.c. of immune serum was again found to be neutralized from 2 to 3 M.L.D. of *Hadogenes* venom in the pigeon. A total of twelve pigeons were used.

TABLE V.

Vol. Serum Horse 394.	Amount Venom in mg. used in Test.	Corresponding Number of Intravenous Pigeon, M.L.D.	Results
1 c.c.	8	1	No symptoms survived
1 c.c.	16	2	Ill, but survived
1 c.c.	24	3	Survived
1 c.c.	32	4	Death after 3 min.
Control	8	1	Death in 15 min.
	9	1	Death in 5 min.

used per test. As in the case of titration on pigeons, neutralization tests on mice were preceded by the determination of the M.L.D. of *Hadogenes* venom by subcutaneous injection into white mice weighing 20 grammes. Examples of such a titration are given in Table VI.

TABLE VI.

Vol. of Serum Horse 394	Amount Venom in mg. used in Test.	Corresponding Number of Subcutaneous Mice M.L.D.	Results.
1 c.c.	35	1	No symptoms survived
1 c.c.	70	2	Death in 20 hr
1 c.c.	105	3	Death in 13 hr
Control	35	1	Death in 7 hr
"	35	1	Death in 6 hr

Repetition of this neutralization test against the same sample of venom and two other pooled samples of *Hadogenes* venom showed that in the case of subcutaneous mice titration, the neutralization power of the serum is found to be comparatively lower than in the case of the pigeon. It is limited to 1 M.L.D. although partial neutralization, reflected in a delay of 12 to 20 hours before death took place, was observed in the mixtures containing 2 and 3 M.L.D. as compared with the control injections of 1 M.L.D. of *Hadogenes* venom, when death occurred 6 to 7 hours later.

No appreciable activity of the serum against the venoms of *Parabuthus* and *Opisthophthalmus* scorpions could be demonstrated, as was to be expected from the limited amount of these antigens used during horse immunization.

#### *Intracutaneous tests in the neutralization of scorpion venom by specific antivenene in the guinea pig*

As shown in previous chapters, the intracutaneous injection of *Opisthophthalmus* and *Hadogenes* venom in the guinea pig resulted in the rapid formation of local haemorrhagic lesions. The following experiments were done with a view to ascertaining whether the haemorrhagic action of these venoms would eventually be modified by anti-scorpion

serum neutralization. Depilated guinea-pigs were injected intracutaneously respectively with:—

1. 8 mg. of *Hadogenes* venom in a volume of 0.3 c.c.
2. 8 mg. of the same venom previously put in contact with 0.2 c.c. anti-scorpion serum for 1 hour—this proportion was found, by subcutaneous tests in mice, to correspond approximately to the neutralization point.

Whereas marked haemorrhagic reactions were observed at the sites of injection in the control animals, reactions of a different character were shown in the guinea-pigs when injected with the same dose of venom plus specific serum. For the dose of 8 mg. of venom alone, the haemorrhagic patch of 20 mm. diameter soon observed at the site of injection, was followed by the formation of a central livid, necrotic zone which became later a hard scab, with permanent loss of hair. In the case of the venom—anti-scorpion serum mixture, the local inflammatory reaction observed soon at the site of the injection did not develop into a necrosis. It remained more superficial as evidenced by the persistent suppleness of the skin and the growth of depilated hairs after 4 days—a sign of the vitality of the deeper layers of the teguments.

The same technique when followed in the neutralization of *Opisthophthalmus* venom produced similar results. The above differences in the reactions produced from the use of venom alone and the use of venom anti-scorpion serum mixtures, were also observed here. The results of these tests tend to show a definite sub-total neutralization of the haemorrhagic principles of these two venoms by specific anti-scorpion serum.

#### PROTECTION AFFORDED BY HETEROLOGOUS ANTI SCORPION SERA AGAINST *Hadogenes* AND *Parabuthus* VENOMS.

##### *Latter Institute anti scorpion serum (M.P.H.).*

Two 1 c.c. ampoules of this concentrated serum (Batch No. 83 date of preparation, June, 1939 date of expiry June 1943) were obtained through the courtesy of Dr. N. KOVACS of the Serum Institute, Cairo. One c.c. was stated to be sufficient to neutralize one sting (species not specified but presumably *Buthus*). 0.5 c.c. of this serum was mixed with 12 mg. of *Hadogenes* venom (1.5 M.L.D. for pigeon intravenously). Soon after the injection, the pigeon showed severe symptoms of intoxication which lasted for several hours, but gradually recovered.

0.5 c.c. of the same serum was mixed respectively with 1 and 4 M.L.D. of *Parabuthus* venom for mice intravenously. The mouse injected with the mixture containing 1 M.L.D. survived, while the one injected with the 4 M.L.D. mixture died in 15 minutes.

Although these tests could not be repeated, due to the limited amount of serum, they tend to show only a limited heterologous neutralization against the two venoms used in these tests.

##### *Institute Enequel Dias (Brazil) anti-scorpion serum.*

One c.c. of this serum (received March, 1941 date of expiry 1945) is stated to neutralize 1.5 lethal doses of *Tityus* scorpion venom for the mouse (camondongo). Neutralization tests on mice using 0.5 c.c. of this serum mixed with 0.2 mg. *Parabuthus* venom (1 M.L.D.) were followed by survival of the animals. Mixtures containing 0.5 c.c. serum with 2 and 3 M.L.D. *Parabuthus*

venom failed to protect the mice. Similar tests were done on pigeons injected intravenously. One c.c. of the serum mixed with 2 M.L.D. (16 mg.) caused the death of these birds in 3 minutes. It does not appear therefore, that anti-*Tityus* scorpion serum confers any appreciable protection against *Parabuthus* venom.

#### ANTI SCORPION SERUM IN THE TREATMENT OF SCORPION STING

Scorpion stings in South Africa although usually very painful and responsible in some cases for severe symptoms of intoxication are seldom fatal even among children whose symptoms are more severe. The fact that no statistical data dealing with scorpion stings or fatalities have been made the subject of special records by the Union Public Health Department, nor by the military authorities, speaks for the relative benignity of scorpionism in this country, as compared with the high death rate reported by TODD (1909) in Egypt. The mortality from scorpion stings in eight great Egyptian towns for the period 1901 to 1907, before the use of anti scorpion serum, ranged annually from 0.036 (in Cairo) to 0.64 per 1 000 (in Assouan). This represents 1.6 per cent of the total death rate in the latter town. Death occurred almost entirely among children. In northern French African territories the severity of scorpion stings inflicted by *Prionurus australis* appears from data given by SERGENT (1940) to be equally grave.

In the absence of any official data for South Africa we have tried to obtain personal information from district surgeons in areas where scorpion stings are particularly frequent. From information kindly supplied by Dr Louw, Brandvlei district, Cape Province we learnt that many cases had been severely ill in this region and some, mostly children, had died. The genus of scorpion found here is usually *Parabuthus*. The main symptom was an immediate and intense burning pain in the area of the sting. If the sting occurred on the leg the pain sometimes extended up to the groin. In 1 or 2 days this was followed by stiffness of the lower limbs and joints and the patient found great difficulty in walking. In some cases the stiffness was generalized and affected the entire body for a few days. These symptoms were accompanied, in some cases by pins and needles in the hands and feet and a pricking sensation in the face. In other cases there was rapid and feeble heart action, while some patients complained of a severe headache and difficulty in talking. In adults this paresis and stiffness gradually passed off in the course of a few days. Usually no outward signs could be found, except some oedema at the site of the sting. Similar symptoms have been described in case records sent by district surgeons in other areas.

The following case record supplied by Dr HOLTZHAUSEN district surgeon, Pofadder, Bechuanaland border of the Cape Province, gives an idea of the condition observed in some patients.



## Cases

A Native mine worker of Caboop Mines was stung by a scorpion on both legs at night during sleep. The mine official was called only on the following afternoon. The patient's condition resembled chronic rheumatism. His arms and legs were drawn up in flexion. His limbs were stiff and muscles hard. He could not talk. In desperation the mine official injected some snake antivenene. The man slowly recovered and 3 days after could walk slowly. The scorpion which stung the Native was sent to us by Dr. HOLTZHAUSEN for identification and proved to be a large *Parabuthus gracilior*.

In children the symptoms are usually severe and may eventually result in death within a few hours as exemplified in the following case reported by Dr. LOUW. Boy of 7 years, was stung under the hollow of the left foot at 7 p.m. Severe pain up the leg and in the epigastrium resulted. Shortly afterwards he became powerless and later paralysed. He could not vomit even when emetics were given. He died at 1 a.m. the following night presumably from respiratory paralysis.

On the whole these symptoms resemble those following stings by *Buthus* and *Priocnemis* as reported by TODD (1909) PHISALIX (1922) and SERGEST (1939) from North Africa.

Critical conditions have been observed in some cases which were stung by *Hadogenes* as illustrated below in the case of a child treated with anti-scorpion serum.

Symptoms following stings by *O. capensis* referred to by EMDIN (1932) are usually not severe—local swelling, redness and pains from 1 to 3 days. Other symptoms are free perspiration, lassitude and sometimes loss of sleep. Fatalities are rare even in children.

#### TREATMENT OF SCORPION STING CASES WITH SOUTH AFRICAN ANTI-SCORPION SERUM.

The anti scorpion serum prepared, as described above, at this Institute was supplied to medical practitioners and district surgeons in the areas where scorpions were reported as frequent or dangerous. Clinical records so far received, on the effects of this serum, although they report favourably on the treatment, are still too limited to have any statistical significance.

It is more difficult to interpret the influence of the serum on adult patients than on children owing to the milder symptoms exhibited by adults. When administered soon after the sting, the serum is reported to minimize the pain considerably and to reduce or inhibit the development of general symptoms. This is exemplified in the following case record supplied by Dr. HOLTZHAUSEN, Kakamas, C.P. —

Patient: European male, aged 40 years, was stung on the hand by a medium-sized *Opisthophthalmus*. Patient's condition was shocked. The site of the sting was burning intensely. Ten minutes later 10 c.c. of anti-scorpion serum were administered in the upper arm and the patient was kept well under observation. One hour later, only slight burning remained at the site of the sting and no other symptoms developed.

Records of cases occurring in children report that the serum had a definite

therapeutic action. The following case was supplied by Dr BOVER Johannesburg —

European child, 12 years old stung at 6.30 p.m. on 15th March, 1943 by a scorpion 2 inches long (apparently *Opisthophthalmus*) on the lateral side of the right knee. The burning pain at the site of the puncture was accompanied by moderate oedema and redness. Next morning there was slight dizziness with local, burning pain on the leg slight oedema, but no necrosis. The child went to school. At 9.30 a.m., during a lesson, the child fainted and was carried out of class. During the following hour he went into one faint after another not totally unconscious, however but could not articulate syllables although he tried to pronounce words. He complained of diffuse abdominal pain. Suffiness was noticed in the hands and fingers. The first clinical examination of the patient by Dr BOVER at 11 a.m. found the patient in a collapsed condition but not completely unconscious, with dysarthria, no excess salivation, pulse normal at 96 heart and lungs normal, respiration regular. He continued to fall into one faint after another. Answers were given with difficulty and in monosyllables, his ideas were confused but his reflexes continued normal. At 1.45 p.m. intramuscular injection of 10 c.c. of anti-scorpion serum was given. At 3 p.m. i.e., 1½ hours later the child became more rational and was able to talk although his ideas were still confused. At 6 p.m. the patient seemed quite normal and awake, speaking without any difficulty. He had no recollection of being carried out of school or of subsequent happenings. The following day the child was normal and his recovery was uneventful.

#### CONCLUSIONS AND SUMMARY

The main toxic and biological characteristics of the venom of the most common genera of South African scorpions have been investigated i.e., *Hadogenes* *Opisthophthalmus* and *Parabuthus*

The average yield of dry venom obtained per specimen was 0.0027 gramme from 9,603 specimens of *Hadogenes* 0.0014 gramme from 5,985 specimens of *Opisthophthalmus* and 0.0048 gramme from 338 specimens of *Parabuthus*

Experimental intoxication with the venom of these three genera was studied on mice, guinea-pigs, pigeons and rabbits. The M.L.D. of the respective venoms for these animals have been determined by intravenous intramuscular subcutaneous intradermal and intracerebral routes. The injection of *Parabuthus* venom by these various routes to the animals resulted in neuromotor symptoms dyspnoea and paralysis ending in asphyxiation. Similar neuromotor symptoms followed the intravenous injections of *Hadogenes* and *Opisthophthalmus* venom, but higher doses were necessary to kill the animals.

The M.L.D. of *Parabuthus* venom by intravenous injection for the pigeon is 0.8 to 1.5 mg. for mice 0.1 to 0.4 mg. and for the rabbit, 5 mg. The intravenous M.L.D. of *Hadogenes* venom for the pigeon is 6 to 8 mg. for mice, 0.2 to 0.6 mg. and for the rabbit, 50 mg. The intravenous M.L.D. of *Opisthophthalmus* venom for the pigeon is 3 to 5 mg. for mice 1 to 3 mg. and for the rabbit, 15 to 20 mg.

The subcutaneous injection of the venoms required considerably higher doses (four to six times higher or more) to produce death. The animals all showed progressive paralysis and prostration ending in asphyxiation after several hours.

The intramuscular injection of doses of *Hadogenes* or *Opisthophthalmus* venom resulted in intense haemorrhagic infiltration followed by sloughing of the necrotic muscular mass and eventual spontaneous amputation of the leg which was injected.

The intracutaneous injection of the venoms of *Hadogenes* and *Opisthophthalmus* into the guinea-pig in doses of 4 mg was sufficient to produce rapid haemorrhagic reactions followed by local necrosis and the formation of hard scabs.

Intracerebral injections of the venoms required doses 25 to 100 times lower than those injected intravenously to kill mice.

M.L.D. of *Parabuthus* for mice is 0.001 to 0.002 mg of *Opisthophthalmus* 0.01 mg and of *Hadogenes* 0.05 mg.

The facts observed point to two main toxic principles in these venoms —  
(a) *Neurotoxin* chiefly developed in *Parabuthus* venom and the cause of the nervous and paralytic symptoms observed in small animals. It appears to be mainly responsible for the fatal cases of scorpion sting in man.

(b) *Haemorrhagin* responsible for the local haemorrhagic, necrotic lesions observed at the site of injection. This was confirmed histologically.

If the relative total toxicity of the three venoms studied is expressed in terms of milligrammes necessary to produce death by intravenous injection in mice of 20 grammes, then *Parabuthus* venom is two times more active than *Hadogenes* venom and ten times more active than *Opisthophthalmus* venom.

A haemolytic action was exerted *in vitro* by the three venoms in very high concentrations in washed erythrocytes. This was more marked with *Opisthophthalmus* and *Hadogenes* venoms than with *Parabuthus*. Guinea-pig red cells appeared more sensitive than sheep cells to haemolysin. No coagulant action was observed with these three venoms on horse blood *in vitro*, but an anti-coagulant action was observed with high concentrations of *Opisthophthalmus* venom. *Parabuthus* venom was found to have a proteolytic action on gelatin *in vitro*, only when relatively high concentrations of venom were used. Heat (1 hour at 80° C) exerted but a limited destructive action on the haemorrhagic principles of the venoms.

No heterologous neutralization of these venoms was observed with polyvalent anti-*Naja flava* *Bitis arietans* serum. A limited heterologous neutralization was found to be exerted by the Egyptian (*Buthus*) and the South American (*Tityus*) anti-scorpion serums.

Immunization of the horse with a view to preparing a specific anti-scorpion serum was started with formalized, detoxicated scorpion venoms, and continued with unaltered venoms. One c.c. of the serum so obtained neutralized *in vivo* 3 M.L.D. of *Hadogenes* venom intravenously injected into the pigeon.

Preliminary results from the use of this anti-scorpion serum in the treatment of cases in South Africa although of no statistical value, indicate that this specific serum has a definite therapeutic value.

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## THE CONTROL OF LEPROSY AMONG THE AZANDE, ANGLO-EGYPTIAN SUDAN

BY

J F E. BLOSS\*

*Senior Medical Inspector Sudan Medical Service*

### INTRODUCTION AND HISTORICAL SURVEY

The high incidence of leprosy among the Azande in the Southern Sudan has been recorded by other observers (CRUICKSHANK, 1932 AtKEY, 1934, 1935, and WOODMAN 1937). The Li Rangu leprosarium was started in 1929 by Dr CRUICKSHANK and since then has become the largest and most important centre of leprosy treatment in the country

The purpose of this paper is to trace the various changes of policy in regard to the disease as it affects this area and to estimate their effect on the disease incidence

Leprosy has existed among the Azande for many years. JUNKER mentions the disease as being quite common in the accounts of his travels about 1870. He noted that those affected had to live in separate huts a distance away from the other people. Nowadays

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\* I am indebted to the Director Sudan Medical Service, Dr E. D. PRIDIE, C.M.D., D.S.O., for permission to publish this paper

the disease is not feared among them, and the leper and his relatives all live together. There is as a result a definite family incidence (35 per cent. among one series of cases).

Towards the end of the first world war a medical commission was sent to this part of the Sudan to investigate the possibility of sleeping sickness having become endemic among the people. Before this date little was known about these people and their country was not fully administered. From 1917 to 1922 sleeping sickness dominated the whole of the medical work. A large sleeping sickness settlement was formed at Temburu and later moved to Sources Yubu. The high incidence of leprosy necessitated the formation of a leper colony as a separate part of this settlement.

In 1929 Dr. CRUICKSHANK carried out a leprosy survey in the Yambio sub-district of Zande country and the leper settlement at Li Rangu near Yambio, was formed.

By the end of 1930 over 3,200 cases had been found in the Yambio sub-district, and 2,800 in the Temburu sub-district. A further 400 were found in the Meridi area. Table I shows the disease incidence as estimated at that time. The estimation of the population is probably very much higher than it should be. The present figure is 180,000, and there is no reason to suppose the numbers have decreased. That would give a leprosy incidence of 3.3 per cent. which is probably more accurate.

TABLE I  
INCIDENCE OF LEPROSY 1931

	Population.	Lepers.	Percentage.
Tembura Yambio	30 000	6 000	4
Meridi	4 500	400	1.8

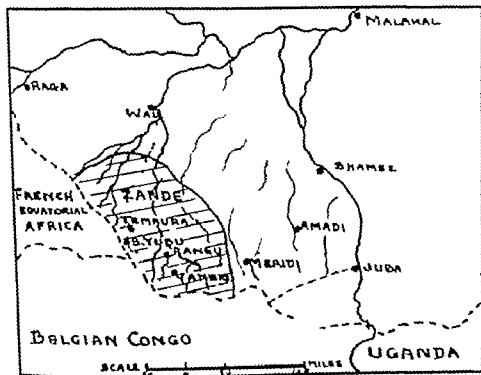
#### PREDISPOSING CAUSES OF LEPROSY AMONG THE AZANDE

The Zande are an agrarian tribe living along the Nile Congo divide. Their total population has been estimated at two million, of whom less than 200,000 live in the Anglo-Egyptian Sudan. Before the advent of sleeping sickness they lived in the forests moving their houses where and when they willed, not cultivating one household plot for more than 2 or 3 years. Sleeping sickness changed this idyllic existence for them and, in the Sudan, from 1920 to 1940, they were made to live along roads and use specified watering places. A census of the population was made to facilitate sleeping sickness work, and inspections carried out monthly or 3-monthly. The disease incidence of the people is thus known fairly accurately.

There are three Zande names for leprosy. One refers to all types of the disease, the second to the nodular form and the third to the mutilated cases. The mutilated cases are those most shunned, though the people have no real fear of the disease in any of its stages, and do not regard it as infectious or contagious.

They ascribe the origin of the disease to some major indiscretion such as eating the meat of one's clan animal. The meat of certain animals is said particularly to bring on the disease especially the bongo (*Boocercus erythrura ussaci*) and wild pig.

By comparison with other tribes in the Southern Sudan they are well fed and starvation is almost unknown among them. Plentiful rains good soil, and an unending variety of forest foods all play their part. Their staple article of diet is cassava (both sweet and bitter varieties are grown though the latter is losing its popularity in some areas). Sweet potatoes eleusine, maize beans ground nuts, earth nuts, sesame and other oil plants are all cultivated easily. Their diet lacks meat in all forms and animal proteins. The only animal that is kept is the chicken and this is more used for their magic rites than for food. This meat shortage is such that any and every form of meat is



Map of Southern Sudan, shewing the Zande area and the main place names mentioned.

devoured voraciously. Rats mice termites locusts and minute bony fish are all relished, while hunting is engaged in on every possible occasion. The tsetse fly prevents their keeping cattle.

The other agrarian tribes living along the Nile Congo border also have a similar diet and the incidence of leprosy among them is also high, particularly in the Raga area. Among the blood and milk eating Nilotic tribes to the north, the incidence of leprosy is low.

In spite of their fat, healthy appearance the incidence of endemic diseases among them is high. These diseases cause little morbidity and the people do not seek treatment for them. For example, ancylostomiasis is as high as 60 per cent. in some areas but anaemias are rare and debility from the disease



not seen. The following figures taken from the examination of some 4,000 school children, recruits, labourers and prisoners indicate the incidence of the more important endemic diseases.

	Per cent.		Per cent.
Ancylostomiasis	40 to 60	Filariasis	70
Schistosomiasis <i>S. mansoni</i>	15 to 20	Skin diseases	10
Veneral diseases	10 to 15	Defective teeth	20 to 30

The incidence of venereal diseases has for long been a problem, and among some classes is as high as 25 per cent. The advent of an administration which affords equal rights to men and women has to some extent undermined parental and familial control but even in the old days the sex life of the average Zande was full and varied.

Routine examination of the blood of prisoners shows that many suffer from the dimorphic anaemias described by TROWELL (1943). The white cell count shows a diminution of the polymorphs often as low as 25 per cent. with a corresponding increase in the lymphocytes. An eosinophilia is common but over 15 per cent. is unusual. Epidemics of measles and influenza have a high mortality rate. Fortunately the more severe epidemics of smallpox and cerebrospinal meningitis have not occurred within the last 30 years.

Of all these factors that of meat shortage is probably the most important predisposing cause of the high incidence of leprosy.

#### THE ORGANIZATION FOR THE CARE AND TREATMENT OF LEPROS, 1929-43.

The two large leper settlements formed at Sources Yubu and Li Rangu in 1929-30 contained some 80 per cent. of the known lepers of the district. The types of cases in them were thus indicative of the types of the disease as found among the Azande.

Of the 5,500 cases in the settlements 54 per cent. were lepromatous, 30 per cent. neural, and 26 per cent. mixed in type.

The age incidence showed that 85 per cent. were between 20 and 40 years, 7½ per cent. under 10 and 13½ per cent. between 10 and 20.

The organization of these two settlements was from the first based on the local native administration. At Li Rangu each outside chief had his own headman or sub-chief inside the settlement. At Sources Yubu there was one chief over the whole settlement. Both settlements had their own native courts, and the doctor was also appointed a magistrate. In these two settlements the doctor in charge was responsible for the planning and layout of the settlement, all the buildings, road making, etc. Each settlement had its own cadre of artisans, usually chosen from the inmates. Native industries were encouraged.

The most effective treatment was found to be alepol, which was given

intravenously (dosage up to 8 c.c. intravenously of a 6 per cent. solution) At the Li Rangu settlement up to 400 injections were being given daily

By the end of 1931 it was reported that 30 per cent. of all cases had improved, 25 per cent. were stationary and 5 per cent. had become worse. The remaining 40 per cent. were considered quiescent, and non-infectious and were then discharged to their outside villages. In this year special segregation camps were built for the advanced lepromatous cases

During the next few years the leprosy work at Sources Yubu suffered as sleeping sickness demanded the whole time of the available medical staff. As a result large numbers deserted to their own homes and only the worst cases remained.

At Li Rangu an attempt was made to bring back as many of the cases as possible into the settlement. Table II shows the distribution of the known lepers in the Li Rangu area for that year

From these figures WOODMAN (*Sudan Medical Service Annual Report 1935*) estimated the disease incidence as high as 7 per cent. of the population, a figure which has not been confirmed by previous or later observers

WOODMAN (1937) records the results of treatment on these 3,500 cases. He found that the chaulmoogra derivatives gave good results if used for a period not exceeding 3 or 4 years. After that period further treatment had no beneficial results. He considered the Li Rangu settlement, then the largest in Africa had been of value in that it had removed the worst foci of infection from outside, and because it facilitated the treatment of cases

By this time the general medical work of the area had increased. A network of dispensaries had been organized throughout the district, which it had been hoped would be able to take part in the leprosy work. Unfortunately lack of suitably trained staff prevented this at that time

The numbers of new cases found each year continued to be large but only the advanced lepromatous cases were now being admitted to the settlement.

From Table II (page 428) it will be seen that 2 176 (62 per cent.) of all cases were nominally living in the settlement. Each of these had their relatives living with them. The area of the settlement was only 25 square miles and the soil not good. The settlement was thus far too overcrowded for an agrarian people. Under such conditions and with inadequate staff the administration of the settlement suffered, and it became unpopular

Sleeping sickness had died out in the Yambio district and the 3-monthly inspections were less well attended. Outside lepers frequently absented themselves or hid and, if discovered, gave another name. Others changed their chiefs and were recorded twice. The records of the outside cases thus soon became muddled owing to the deceit of the wily African. The people realized that the treatment of leprosy was not so efficacious as they had hoped. Many refused to come in to the settlement and others deserted and have never since been traced.

By the end of 1938 there were over 6 000 people still living inside the Li Rangu settlement. Of these less than 1,500 were lepers, the remainder were in some cases relatives, but there were also many who sought refuge there to avoid their civic duties to their outside chiefs.

In 1939-40 a new survey of the known lepers in the district was carried out. This was helped by the compilation of a new census of the population. The total numbers seen at sleeping sickness inspections then rose from 45,000 in 1938 to 60 000 in 1940. The whole area was being inspected once yearly so this figure gives an approximation of the total population. It was estimated that from 5 to 10 per cent. could be considered as absentees who were never seen. As in the past, at each inspection a special inspection was made for lepers. This complicated the old records as in an effort to evade being recorded as a leper many gave wrong names or changed their chief. These difficulties were overcome by the end of 1941 by a card index system, and by more accurate

TABLE II  
LEPERS, LI RANGU (YAMBO DISTRICT), 1935.

A.	Advanced cases (segregated)	..	...	...	...	704
B.	Under observation or treatment in settlement	...	...	...	...	1,821
C.	Discharged cases. Cured and quiescent, still living in settlement	...	...	...	...	831
D.	Discharged cases. Living outside settlement	..	...	...	...	813
E.	Outside cases, who had never been in settlement	...	...	...	...	879
Total						2,933

registration of the individual cases. The numbers of "new" cases then fell, and the numbers of absentees were less than 5 per cent. in most chiefdoms.

The cases were then divided into three main groups.

1 *Segregated Cases*—The most advanced lepromatous cases, or those which were most "infective" and the mutilated cases. These were classed together for purposes of care and treatment.

2 *Settlement Cases*—These lived inside the Li Rangu settlement with their relatives under conditions approximating their life outside. These were under treatment or observation for special reasons.

3 *Outside Cases*—Living in their own villages and having no treatment.

Table III shows the numbers of each group for the years 1939-42.

The total of 2,931 known lepers on the records at the end of 1939 shows a drop of over 550 from WOODMAN's figures of 1935. This is explained by reduplication of records, desertions, cures, and deaths that had been discharged from the records in those years. All records were kept for a reasonable period before any leper was crossed off the register as a "deserter" or "died."

A further drop of 300 cases is noted for the next year. This is explained by the discharge of further cases from the records as deserters or "cures," mostly from those in sections (C) and (D) in Table II.

By 1941 the new card index had been completed for all the cases which could be traced, and only a few of those on the old records were still left unknown. These were then finally crossed off the records in 1941, together with all the 'cures' and cases without any visible lesion who had been seen for the last 3 years.

In 1942 a further 230 cases were crossed off the records as cases which had had no visible lesion for 3 years. Of these 189 were early  $N_1$  cases or doubtful cases of leprosy who had been under observation for this period. Thus the statistics for 1942 show all the known lepers with any definite lesion.

Tables IV to VIII (pages 430-34) show the detailed statistics for the year 1942. The classification of cases follows that of the International Congress on Leprosy held in Cairo in 1938.

TABLE III  
LEPERS LI RANGU (YAMBIO) DISTRICT 1939-42.

	1939	1940	1941	1942.
Segregated	181	212	200	178
Settlement	1 183	950	625	593
Outside	1 597	1 455	1 130	908
Total	2,961	2 617	1 955	1 679
New cases	283	241	186	86

From the above it will be seen that the compilation of accurate statistics is indeed difficult even where the people are known and their living areas are controlled as necessitated by the boggy of sleeping sickness. The tables for 1942 are a true picture of the incidence of the disease in that year as they include the known cases, and not large numbers who have not been seen. To include every case that has ever been recorded, but which has not been traced merely falsifies the picture. These figures cover 60 000 of the population.

During the same period the Li Rangu settlement was reorganized. A new segregation camp was made, including a dispensary with leper wards, wells, latrines and a general meeting house for school, court or religious services built. In this camp the advanced and more infectious cases had to live, while their relatives were put on a road round this camp and about three-quarters

of a mile away. These lepers were provided with daily rations, including meat, as was given to hospital patients. Clothes were issued regularly and all were under treatment. This improvement in their living conditions reduced the number of absentees. At certain times of the year *i.e.* for hunting expeditions, or during the early rains when the termites were in flight, they were allowed out. Such a camp should not be regarded as a prison or it fails completely. Some reasonable freedom of movement is essential.

TABLE IV  
ORGANIZATION LEPERS LI RAMOU BY TYPE 1941

Type of Cases.	Outside Cases.	Settlement Cases.	Segregated Cases.	Total.	Under Treatment.	New Cases.
L <sub>1</sub>	69	70	1	140	43	
L <sub>2</sub>	12	7		21	8	3
L <sub>3</sub>	2	1	6	9	7	
N	686	231		917	49	63
N	27	49		56	12	2
N	4	14	7	25	16	
L <sub>1</sub> N	64	74	1	139	53	9
L <sub>2</sub> N	9	79	7	95	63	
L <sub>3</sub> N	4	47	20	51	38	
L <sub>1</sub> N	1	20	11	22	6	2
L <sub>2</sub> N	6	26	16	48	36	1
L <sub>3</sub> N		10	29	31	43	
L <sub>1</sub> N	1	2	17	20	19	1
L <sub>2</sub> N	1	1	22	24	23	
L <sub>3</sub> N			19	19	19	
Total	908	593	178	1 679	463	66

Classification of type f cases according to the International Congress on Leprosy held in Cairo 1938.

In the past the settlement lepers worked 1 day a week for the settlement without pay. This was stopped in 1940 and the agricultural side of the settlement was developed. These lepers (settlement cases) worked on the cultivations and were paid the ordinary daily wages. This provided the settlement with almost all the food crops necessary even though the rations for all had been increased four or five times, and in addition improved the general hygienic of the settlement. Such agricultural development was carried out on a proper basis with the aid of the Agricultural Inspector. Half of these cases were under treatment. (See Table VII)

From the statistics it will be seen that the numbers in the settlement

have been reduced considerably. This, together with the all round improvement in their living conditions reduced the unpopularity of the settlement. Treatment has been limited to numbers that the staff can cope with, and attention has been paid to training staff who it is hoped in the future will be able to work in the district dispensaries later.

Treatment has on the whole been disappointing and there is little evidence in favour of any one particular line of treatment or of one particular drug.

TABLE V  
OUT LEPROS, LI RANGU (YAMINGO) DISTRICT 1942.

Type of case.	Progress of Old Cases							Total Old Cases.	Total No Cases Discharged.	New Cases.	Total remaining
	Im proved.	Worse	Sta tionary	Cures.	De serted	Died	Ab-sentees not seen				
1	8	2	31	17	8	1	8	95	26		69
1	3		4		3	1	2	13	4	3	12
1		2						2			2
1	177	31	335	123	38	13	75	801	183	68	686
1	5	7	11		4	2	2	31	6	2	27
1			3			1	1	5	1		4
N <sub>1</sub>	17	6	24	13	1	1	8	70	15	9	64
N <sub>1</sub>	1	5	2	1	1	2	1	13	4		9
N <sub>1</sub>		2	2		1			5	1		4
N <sub>1</sub>	7	3	5		3		4	22	3	2	21
N <sub>1</sub>		1	3		2		1	7	2	1	6
N <sub>2</sub>			1				1	2			2
N <sub>2</sub>										1	1
N <sub>2</sub>			1					1			1
total	238	59	422	163	61	21	103	1067	245	86	908

In all cases the first thing has been to treat any endemic diseases such as ancylostomiasis or intestinal bilharziasis. The improvement of rations with a regular supply of meat has also been considered of prime importance for the segregated cases. Specific treatment has been limited to the drugs available during war time. Creosoted hydnocarpus oil has been used as a routine when available, and alepol was used for a short time when this ran out. Both these drugs have been used as intramuscular and intradermal injections. Tuberculoid cases of doubtful activity have been treated with a 25 per cent. trichloroacetic acid paint. Cures, or improvements occur with equal proportion in

those under treatment and those without treatment. It must, however, be remembered that the worse cases were put under treatment, and that the milder cases were not under treatment. The results of treatment on the settlement and segregated cases are tabulated in Tables VII and VIII. Table IX gives the types of cases classed as "cures" for the years 1940-42. All these latter cases have been under observation for a period of 3 years or more, and for that period have had no visible lesion. These have not been included in the

TABLE VI.  
PROGRESS OF ALL SETTLEMENT LEPROS, 1941.

Type of Case.	Progress of Old Cases.							Total Old Cases.	Total Cases Discharged from Settlement.	New Cases.	Admitted to Settlement.	Total remaining.
	Improved.	Worse.	Stationary.	Cures.	Discharged.	Died.	Absentee-not seen.					
L <sub>1</sub>	11	3	41	9	-	2	6	76	4	-	-	29
L <sub>2</sub>	5							7				1
L <sub>3</sub>		1						1		-	-	1
N	41	14	113	57	7	1	9	45	8	-	-	236
N	9		17			1	1	22	2	-	-	29
N	3	4	4		1		3	15	1	-	-	14
L <sub>1</sub> N	22	12	24	1	1		5	75	1	-	-	7
L <sub>2</sub> N	19	1	22		3		7	84	6	-	-	27
L <sub>3</sub> N	4	10	10		1	3	3	31	4	-	-	20
L <sub>4</sub> N	8	2	6				4	20		-	-	23
L <sub>5</sub> N	9	9	7				1	26		-	-	19
L <sub>6</sub> N	5	4			1			11	1	-	-	1
L <sub>7</sub> N										-	-	
L <sub>8</sub> N		1						1		-	-	1
L <sub>9</sub> N										-	-	
Total	136	63	266	67	18	9	39	620	-	-	-	295

final statistics for the end of 1942. Their case records have been kept for reference in the case of a relapse.

One of the main difficulties in an area such as this where the disease incidence is high is a tendency to diagnose any and every skin blemish as a case of leprosy or as a precursor of leprosy. Only the definite cases have been included in the later statistics. It is admitted that some probable cases have thus been left out, but this is better than to include large numbers of doubtful cases and so falsify the disease incidence.

It is thus claimed that these statistics covering some 60 000 of the people, give as accurate as possible the incidence of the disease for 1942. These give an incidence of less than 3 per cent. (making due allowance for cases not seen) This is approximately the same as that recorded by CRUICKSHANK in 1930

A study of the age incidence of the disease shows that less than 12 per cent. of the known cases are under 20 years of age, and that new cases among children are comparatively rare. In 1930, 21 per cent. of all cases were under 20 This

TABLE VII.

PROGRESS OF SETTLEMENT LEISERS WITH AND WITHOUT TREATMENT 1942.

A.—Under Treatment.						B—No Treatment.					
Type of Case.	Im- proved.	Worse.	Sta- tionary	Cures.	Total Under Treat- ment.	Type of Case	Im- proved.	Worse	Sta- tionary	Cures.	Total
L <sub>1</sub>	10	2	29	1	42	L <sub>2</sub>	1	1	12	8	22
L <sub>1</sub>	4		2		6	L <sub>2</sub>	1				1
L <sub>1</sub>		1			1	L <sub>2</sub>					
N <sub>1</sub>	22	5	21	1	49	N <sub>1</sub>	19	9	92	56	176
N <sub>1</sub>	4	2	6		12	N <sub>1</sub>	5		11		16
N <sub>1</sub>	2	3	4		9	N <sub>2</sub>	1	1			2
L <sub>1</sub> N <sub>1</sub>	19	10	23		52	L <sub>1</sub> N <sub>1</sub>	3	2	11	1	17
L <sub>1</sub> N <sub>1</sub>	16	14	25		55	L <sub>1</sub> N <sub>2</sub>	3	7	7		17
L <sub>1</sub> N <sub>1</sub>	4	7	7		18	L <sub>1</sub> N <sub>2</sub>		3	3		6
L <sub>1</sub> N <sub>1</sub>	6	2	6		14	L <sub>2</sub> N <sub>1</sub>	2				2
L <sub>2</sub> N <sub>1</sub>	8	8	4		20	L <sub>2</sub> N <sub>1</sub>	1	1	3		5
L <sub>2</sub> N <sub>1</sub>	3	3			6	L <sub>2</sub> N <sub>2</sub>	2	1			3
L <sub>2</sub> N <sub>1</sub>		2			2	L <sub>2</sub> N <sub>1</sub>					
L <sub>2</sub> N <sub>1</sub>		1			1	L <sub>2</sub> N					
L <sub>2</sub> N <sub>1</sub>						L <sub>2</sub> N					
Total	98	60	127	2	287	Total	38	25	139	65	267

points to (a) the disease being under control, and (b) that a decrease rather than an increase in the number of cases is to be expected in the future. This is the brightest side of the picture

#### THE OUTLOOK FOR THE FUTURE.

This part of the Anglo-Egyptian Sudan is still in a very early stage of development. It is however that part which gives most promise for economic development in Equatoria Province. Various schemes have been proposed, but these have of necessity been held up for the duration of the war



With this economic development it is hoped that the lot of the native will improve. The meat shortage should be alleviated, and other commodities of civilized life be more readily available for all.

While there should be no attempt to minimize the leprosy problem in the area, at the same time it should not be exaggerated. If anything the disease is on the decrease, and at least it is under control.

The work done in the past has been rather disappointing. Leprosy and

TABLE VIII  
PROGRESS OF SEGREGATED LEPROS, 191...

Type of Case.	Progress of Old Cases.							Total Old Cases.	Total No. Cases Discharged to Settlement.	Total number
	Im proved.	Worse.	Sta- tionary	Curra.	De- seried.	Dyed.	Abs- entees not seen.			
L <sub>1</sub>			1					1		1
L <sub>2</sub>			1				1	2		2
L <sub>3</sub>			3				2	5		5
N										
N			5			1		6	1	7
L <sub>1</sub> N							1	1		1
L <sub>2</sub> N	3		3		1	1	1	9	2	11
L <sub>3</sub> N	8	2	8			1	1	21	1	22
L <sub>4</sub> N	5		3		1		3	12	1	13
L <sub>5</sub> N	7	4	4			1	1	19	3	22
L <sub>6</sub> N	15	2	18		1		4	40	1	41
L <sub>7</sub> N	5	3	6		2		4	19		22
L <sub>8</sub> N	9	11	10		1	3	2	35	3	38
L <sub>9</sub> N	3	7	8		1		1	20	1	21
Total	58	27	70		9	6	22	192	15	207

sleeping sickness both altered the normal life of the native, and caused many irksome restrictions to their habits and customs. Both these diseases being under control, it has been possible in the last few years to remove most of the restrictions.

In any public health campaign the first essential is education. This serves two purposes. On the one hand it provides better personnel for training for anti leprosy work, and on the other it enables medical propaganda to reach a larger proportion of the population.

I have always maintained that the other endemic diseases affecting so large a proportion of the population should receive first attention. Once these have been reduced and education increased, leprosy would automatically die out. Leprosy is a disease of low standards of living. Specific treatment has little effect on the incidence of the disease. Leprosy must always be viewed in its proper perspective.

The large leprosarium and compulsory segregation have not been really successful in coping with the problem in this district. It is generally accepted that this policy is neither desirable nor necessary. Small local settlements for the bad and infective cases with some form of out patient treatment is much more the ideal. But among such primitive peoples the larger settlement has its place until sufficient staff have been trained to work in the local or chiefs' settlements. Again these chiefs' settlements to be successful, must have

TABLE IX.  
RECORDED "CURES" 1940-1942.

Type of Cases		$L_1$	$L_2$	$N_1$	$N_2$	$L_1N_1$	$L_1N_2$	$L_2N_1$
1940	Outside cases	61	7	104	—	8	—	3
	Settlement cases	6	—	93	1	—	—	—
1941	Outside cases	112	3	202	4	1	—	4
	Settlement cases	5	—	36	—	—	—	1
1942	Outside cases	17	—	132	—	13	1	—
	Settlement cases	9	—	57	—	1	—	—

intelligent and full support both of the chief and his people. Such support can only be obtained from a highly educated native administration of long experience. This will come in the fullness of time in the Zande area, but to force it at the wrong time would create more chaos.

The present policy of keeping as many as possible of the active cases and cases that might benefit from treatment in one large settlement, must remain purely a temporary one until the chiefs' colonies can be formed. This policy has at least controlled the disease and probably caused a decrease.

For the future the gradual decentralization of leprosy work is being aimed at. Coupled with this is an increase in the public health and general medical work in the area. By these means it is hoped to raise the standards of living and so gradually eliminate the disease.

## SUMMARY

- 1 A survey of the history of leprosy and its predisposing causes in the part of the Sudan has been made.
- 2 The incidence of the disease as recorded for the past 15 years is discussed, together with the various methods of control that have been used.
- 3 The effectiveness of this policy is discussed, with recommendations for the future.

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## KWASHIORKOR AND ARIBOFLAVINOSIS

BY

WILLIAM HUGHES M.D., M.R.C.P.\*

*African Hospital Lagos Nigeria*

Although the first adequate clinical account of kwashiorkor was provided by WILLIAMS (1933) the reviews of STANNUS (1936) and TROWELL (1941) make it clear that a similar condition was recognized independently and described by other observers in East Africa (PROCTOR, 1926 GILLAN 1934) and the Congo (LIEURADE, 1932, TROLLI 1938 VAN DAELE, 1938) about the same time. The diverse nomenclature, "Gillan's oedema, cheveux blancs," "enfants rouges," "kwashiorkor" 'infantile pellagra', reveals the prejudice of each observer in favour of what he considered either the most prominent symptom or the most likely cause.

A study of deficiency in Lagos during the past 4 years has led us to recognize on an indeterminate background of general malnutrition three rather well-defined syndromes, *viz* simple ariboflavinosis, kwashiorkor and nutritional achromotrichia. This is not to say that all forms of local malnutrition can be grouped under one or other of these headings—indeed, the commonest expression of malnutrition is a retardation in physical development with few or no signs of specific deficiency. The position is that in each of these states there is typically a specific group of symptoms and lesions which suggest that we are dealing with a constant deficiency. Typical cases in each syndrome are hard to define and it is probably unwise to enforce a rigid classification.

Simple ariboflavinosis is the condition in which we see only those lesions of the tongue, mouth and external genitals characteristic of the adult syndrome. Such cases are rare in children but they have an importance out of proportion to their incidence because they constitute a link between the adult syndrome of ariboflavinosis and kwashiorkor. We see depigmentation of hair in some cases of kwashiorkor but alopecia rather than achromotrichia is the distinctive lesion. Nutritional achromotrichia appears to be a distinct syndrome in which the outstanding feature is depigmentation of the hair. The muco-cutaneous lesions of kwashiorkor are minimal or absent. Examples of the pure syndrome are rare. So far as our observations go they suggest that nutritional achromotrichia is the result of pantothenate deficiency. Achromotrichia as

\* I am indebted to the Pathological Department of the African Hospital, Lagos, and particularly to Dr R. B. T. BALDWIN for much help. I have to thank the Director of Medical Services, Nigeria, for permission to publish this paper.

a symptom of kwashiorkor seems to be more common in the Congo than elsewhere and probably accounts for the nomenclature in that area.

This paper deals with observations on sixty five cases diagnosed as kwashiorkor within the past 4 years in the children's ward of the African Hospital, Lagos. On the clinical side there is little to add to the accounts which have already appeared but the recent advances in our knowledge of the B<sub>2</sub> complex deficiencies give many of the symptoms and lesions a new significance.

#### DIETETIC AND SOCIAL BACKGROUND.

Our knowledge of the dietetic background is fragmentary both from the quantitative and qualitative points of view. One thing is clear however the deficiency or deficiencies concerned do not depend on the staple alone. Kwashiorkor has been recognized in different areas in which the staple has been cassava, yam, plantain, maize, millet and rice. In Southern Nigeria yam and cassava are the staples. Small amounts of guinea-corn, rice, groundnuts and fruit are consumed but we have no exact quantitative data about these ingredients. In the case of the soup or stew we know the amounts of the most expensive ingredients such as meat and fish are restricted for the poor and the variety is restricted in towns generally but again exact data are lacking. The cooking fat is red palm oil. Increasing attention is being paid to the "unponderables" of the diet among which the preparation and method of cooking are of the highest importance. On the positive side, we have various fermented foods, not only such obvious sources of yeast as native beer and palm wine but also cooked foods which are left lying about to mature for anything up to a week according to the vagaries of native custom. Although objectionable from the hygienic standpoint, such treatment by encouraging fermentation will enhance the vitamin B-complex content of the food. On the negative side, however we have the custom of boiling the green vegetables in water, taking them out and squeezing them and then returning the squeezed product only to the soup. This must result in loss of appreciable quantities of water soluble vitamins. It will obviously take many years of field work and laboratory study to work out the exact constituents of the local diet as it is consumed, and assess the merits and drawbacks of the local methods of cooking.

As WILLIAMS has noted, it is typically a disease of the child who is neglected or deposed from the breast before the next confinement is due. Weaning is usually a gradual process extending over 1 or 2 years when possible. When, however another confinement is due the child may be switched over abruptly to "pap" and soup. "Pap" is a gruel made from guinea-corn. In six of our cases the diet has consisted of pap alone—in one case for 2 months before admission. The disease is frequently seen in twins—five pairs were noted in the series. In six instances the child was motherless. In many

instances, with breast-fed babies the mothers have shown the oral lesions of ariboflavinosis. In one case the mother had filariasis of the breast.

The incidence of clinical ariboflavinosis is high among the local adult population the characteristic lesions of the tongue lips and external genitals being particularly evident among the out-patients and in-patients at the African hospital and in the inmates of the local jails and asylums. From July 1944 to July 1945 all the new admissions were examined on entry to Lagos prisons. The incidence of ariboflavinosis was found to fluctuate from 9.5 to 20.2 per cent, giving a rate of 16 per cent. in the total examined. Most of the admissions were petty criminals from the poorest section of the community—the same source from which the majority of our kwashiorkor cases derive. This high incidence of ariboflavinosis is the most outstanding feature in the social background of the disease. The plight of children in the community is aggravated by the universal social custom which ordains that the wage-earner shall have the choicest bits from the communal soup.

#### CLINICAL HISTORY

A history of enteritis or dysentery is very common. In the later stage however the diarrhoea usually stops and only an occasional white or clay-coloured formed stool may be passed. This, no doubt, is conditioned partly by the fatty infiltration of the liver which is so intense in severe cases and partly by dehydration. Before we became familiar with this change in the stools we believed that diarrhoea was not an essential factor in the syndrome. On closer investigation, however a history of dysentery or enteritis was established in sixteen out of twenty two cases treated in 1945. Four cases had abdominal tuberculosis (all fatal) one lobar pneumonia with recovery three major staphylococcal lesions (two fatal). Helminthiasis and malaria are contributory but not essential factors. Many severe cases showed no ova in the stool. Lack of absorption and the increased metabolic demands of infectious disease obviously combine to precipitate the deficiency in many cases.

#### AGE LIMITS.

Our cases were distributed in age groups as follows. 0 to 1 year fifteen 1 to 2 years, fifteen 2 to 5 years twenty seven and 5 to 9 years, eight. The mortality and severity of the disease decline with advancing age. From the age of 8 upwards we meet fewer cases of the kwashiorkor type and more and more of the adult ariboflavinotic syndrome. Between the ages of 8 and 16 years we meet transitional cases in which the distribution of the skin lesions still corresponds with kwashiorkor but the intensity of the exfoliation is progressively diminished. In such adolescent cases depigmentation has been more intense than exfoliation. This type of case appears to be more common in the Cameroons and Congo than further north. Whether it depends on a

primary dietary deficiency or is a sequel of the chronic parakeratosis it is impossible to say.

#### EPITHELIAL CHANGES.

The typical case of kwashiorkor includes the lesions of the tongue, lips and external genitals which correspond to those of the adult syndrome of ariboflavinosis. The tongue may be pink or magenta but the change in colour is often obscured by anaemia, thrush or dehydration. The papillae are enlarged but not to the same extent as in adults nor are fissures present. In dehydration it is glazed and pointed and the enlargement of the papillae accentuated. The specific changes may disappear overnight as the result of treatment. The lips show desquamation and may be red or vermillion and fissures are common at the centres and angles. The angles may be ulcerated but rarely do we see the white heaped-up sodden epithelium so frequent in adults. The lesions of the external genitals are much more severe in children, and especially in female children than in adults. At first the rash shows the same hyperpigmented scales on a hypopigmented background involving the scrotum and prepuce in males and the vulva in females. Later all skin from the pubis to the coccyx and for an inch or more beyond this becomes raw moist and inflamed. Fissures develop between the penis and scrotum and about the glans in males and alongside the vulva in females. The condition in females can be mistaken for gonococcal vulvo-vaginitis.

It is the extension of the rash on to sites other than those affected in the adult syndrome that gives to kwashiorkor its chief characteristic. It occurs particularly on the extensor surfaces of the limbs and is most marked on extremities—the dorsa of the hands and feet, the scrotum, the prepuce or tip of the glans penis, the tip of the nose the tip of an umbilical hernia when present the pinnae of the ears. The desquamation on these sites is clinically identical with that seen on the adult scrotum but the exfoliation is often more intense. On the dorsa of the hands and feet large blisters may form. The intensity of the process is also evident in the face where the seborrhoeic lesions of the adult syndrome are often accentuated by deep fissures at the canthi, naso-labial sulci and above the ears. Depigmentation and fissuring are severe in the flexures—axillae, groins and popliteal spaces. The typical crumbly pavement desquamation, about which so much has been written, occurs just as the hyperpigmented epidermis cracks for the first time and the scales are about to separate. The phase is evanescent as the continual exfoliation leads to depigmentation and the contrast is lost. In chronic cases nothing more than an irregular mottling or powdery desquamation is noted.

Superficial erosions and "septic spots" are common. I have seen an erosion develop on the lower lip in a few hours through the misguided efforts of an attendant to feed a semi-comatose child. Two of our cases died from disseminated staphylococcal infection.



FIG. 1—Adult arboflavmosis showing characteristic lesions at angle of lips, naso-labial sulcus and external canthus



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FIG. 3—Adult ariboflavinosis. Male external genitals

FIG. 4—Kwashiorkor Unusually severe depigmentation in groin and at tip of prepuce.

FIG. 5—Kwashiorkor Female external genitals, showing intense exfoliation.

FIG. 6—Kwashiorkor Female external genitals. Note how ariboflavinotic exfoliation and depigmentation on vulva and perineum merges with the "pellagroid" dermatitis on thighs.



7



8

FIG. 7.—Kwashiorkor. Wasting of buttocks, erosions, desquamation and alopecia on 15.12.44

FIG. 8.—Same case after treatment—4.4.45.

Microscopically sections show the condition to be one of parakeratosis. There is a thinning out of the epidermis with acanthosis and a hurried maturation and cornification of the epithelium. Nucleated cells containing pigment are seen passing into the *stratum corneum* which is semi-detached. Many of the epithelial cells are vacuolated. In the tongue there is similar histological picture. Acanthosis is marked. Vaso-dilatation and extravasation of red cells were noted in our sections but it is difficult to say how far such findings were abnormal in such a vascular organ as the tongue.

The hair tends to fall out and in severe cases it can be combed or pulled out painlessly in large bunches at a time. In chronic cases there is loss of pigment but this is not an essential feature of the acute disease.

Clinical and histological appearances suggest that there is no essential difference between the epithelial changes in kwashiorkor and adult ariboflavinosis. It is merely a question of intensity and distribution. One might compare the histological changes in the skin in kwashiorkor with those in the bone marrow in pernicious anaemia. In pernicious anaemia the long bones are filled with red marrow which fails at the last step to turn out mature erythrocytes. In kwashiorkor the march of cells from the basal layers to the surface is accelerated. This hyperplasia results in a loss of pigment and immature cells from the semi detached cornified layer. The manufacture of pigment and cells cannot keep pace with the demand and depigmentation and erosions are the consequence. In kwashiorkor and in pernicious anaemia the consequence of deficiency it should be noted, is not atrophy but hyperplasia. The age factor may account for the more intensive and extensive epithelial changes in kwashiorkor compared with the adult ariboflavinotic syndrome.

#### LETHARGY AND PRE TERMINAL COMA.

Although some cases are irritable in the beginning as WILLIAMS noted, all drift into a peculiar state of lethargy near the end. This state resembles surgical shock but the patient can be roused and has been known to speak intelligently half an hour before death. Usually the child passes from this stage gradually into coma but in some cases the transition from apathy to coma has been abrupt and unexpected. In four cases death occurred unexpectedly during the night or early morning probably after a brief period of coma. SERRELL (1929) originally described this sudden lapse into coma quickly followed by death in dogs deprived of riboflavin.

#### MUSCULAR WASTING

Muscular wasting is an essential feature of the disease. It may be masked in the beginning by oedema. The buttocks are often grossly wasted and the spine become kyphotic from wasting of the erectors. Prolapse of the anus was noted in four cases. It is probably due partly to the muscular atrophy and partly to tenesmus.

The mortality in Lagos during my last three tours has been as follows 1942-43 nineteen cases with eleven deaths 1944 twenty four cases with fourteen deaths 1945 twenty two cases with eleven deaths.

The type of case has not varied between 1942 and 1945. Although there has been some improvement in our results it is clear that we are coming up against a "hard core" of mortality consisting of (a) cases in which the changes in the liver are irreversible—or at least not amenable to riboflavin and (b) those in which the concomitant disease is beyond our control. In the following analysis of the 1945 mortality I have sketched in briefly the outstanding clinical and pathological findings which were noted in addition to the kwashiorkor syndrome in each case.

1. Admitted moribund, died within 24 hours. had no riboflavin or liver extract fatty liver postmortem.
2. One of breast fed twins enteritis for 7 days. Her sister showed the typical oral and genital lesions of ariboflavinosis. The mother had cheilosis and glossitis mother and sister cleared up within a week on oral riboflavin. The patient had 2 mg. riboflavin and 2 c.c. liver extract died within 24 hours.
3. Died within 48 hours. We had run out of liver extract and riboflavin for injection.
4. Bacillary dysentery. Lived 4 days (see below).
5. Lived 4 days. riboflavin 5 mg. i.m. Fatty liver postmortem.
6. Subtertian malaria and enteritis. Lived 5 days. had only 2 mg. riboflavin by injection.
7. Seemed to be making good progress after a single injection of 10 mg. riboflavin, but died unexpectedly in the early morning of the 6th day. fatty liver postmortem.
8. Motherless infant, 21 days old. Was recovering but developed intestinal obstruction of unknown causation and died after 7 days.
9. Severe enteritis. "Plexan," 2 c.c., and riboflavin, 2 mg., injected on 6th day; died 7th day.
10. Lethargic on admission. passing occasional grey stool. had 20 mg. riboflavin by injection daily. died 3rd day. fatty liver postmortem.
11. Lethargic on admission. initial improvement after 10 mg. riboflavin i.m. in spite of 20 mg. oral riboflavin daily. skin lesions relapsed and coma supervened on 8th day. Liver fatty postmortem.

The details supplied in these cases illustrate the difficulties in arriving at clear-cut conclusions in an investigation of this kind. No. 2 illustrates our conception of the origin of kwashiorkor. The mother had ariboflavinosis and both breast fed twins were affected equally until one developed enteritis. Was death due to enteritis or acute riboflavin deficiency? Was fatty liver the fatal lesion? Were the changes irreversible or would a larger dose of riboflavin have been effective? Was death due to some other deficiency—protein, ash, methionine, nicotinic acid, pantothenate? These are the questions prompted but only partly solved by a study of our successes and failures to date.

No. 7 illustrates a mode of death already described—a sudden and unexpected lapse into coma followed by death in a matter of hours. The most dramatic instance was seen in a case which I demonstrated to a class of students. The rash and oedema were extensive but the child sat up and answered questions intelligently. Although I described this mode of death to the class as part

of the kwashiorkor syndrome, it came as a shock to find that the child died during the early hours of the following morning.

It might be urged in the cases of Nos. 4, 5, 6, 8 and 9 that the true cause of death was the concomitant disease. The exact relationship between the concomitant disease and kwashiorkor and the contribution of each to the high mortality is difficult to define, but it is believed that in all eleven cases the child would normally have died from kwashiorkor. This view makes the results of our treatment appear worse than they really are but it does emphasize the fact that we cannot abstract a part of kwashiorkor from its clinical background and hope for perfect results when we have dealt with the ariboflavinosis alone. The clinical notes of No. 4 might be amplified to demonstrate this —

*A A—Motherless female, aet. 4 years.* Admitted 23.1.45 with history of diarrhoea for 1 month. On admission lethargic with generalized oedema and extensive exfoliation and depigmentation on limbs, buttocks, abdomen and face; tongue red and glazed; lips red, angles ulcerated; blepharitis with scaling and fissures at canthi; vulva very eczematous with fissures. No diarrhoea; stool formed, white.

Treatment. 23.1.45 Saline 150 c.c. intraperitoneally. Riboflavin 10 mg. i.m. and 20 mg. orally repeated daily.

25.1.45 Improving. Lips and angles cleared up. Blepharitis almost healed; vulva improved but still red.

26.1.45 Diarrhoea with mucus very weak. Saline repeated. Aneurin, 5 mg. and "Plexan" 8 c.c. i.m. Died that day.

Stool *E. coli* cysts and cellular exudate; culture—no dysentery organisms isolated. Postmortem: Injection of terminal ileum and colon. Liver—a few fat globules at periphery of lobule.

The clinical findings, including the white stools, suggest that the liver was severely affected in the beginning but improved with treatment. Death was probably due to bacillary dysentery.

We have found the first 4 days after admission are the critical period and it was during this time two specific effects are noted. Firstly the epithelial lesions of the tongue, lips and external genitals showed signs of clearing up within 24 hours of giving riboflavin by injection. Secondly an improvement in appetite seemed to be a specific effect of the liver extract injections and was often evident in 24 to 48 hours.

A favourable response to treatment and progress is illustrated in the following cases —

*Male, aet. 5 years.* Admitted 12.12.44. History of wasting—2 months, no diarrhoea. On examination, 13.12.44 lethargic with generalized oedema and wasting; many erosions especially over bony prominences; deep fissures behind right ear and in perineum; smaller fissures at angles of mouth and external canthi and between fingers; tongue appears normal, lips red with central fissure; scrotum and tip of umbilical hernia glazed; generalized depigmentation with hyperpigmented scaling. Hair dark, straight, falling out; blepharitis. Not passing stool or urine. B.P. 92/66. Blood few *P. falciparum*.

Treatment. 12.12.44 Riboflavin, 12 mg. i.m. and nasal feeds of milk. Riboflavin 10 mg. daily till 16.12.44.

14.12.44 Epithelial lesions improved; has passed a grey stool and some urine (no albumen). More irritable.

15.12.44 Much improvement. Lips and angles healed. Blepharitis cleared up. Fissures healing rapidly (see Fig. 7). Oedema going down.

16.12.44 Taking food orally Erosions much improved.

28.12.44 Skin lesions cleared up Can sit up. Very emaciated.

Progress thereafter uneventful. The photographs (Figs. 7 and 8) illustrate the condition on 15.12.44 and on 4.4.45.

The above was one of the few cases in which riboflavin alone was given. In most cases crude liver extract was given as well, and the combination seemed more efficient especially in the matter of restoration of appetite. In many instances the treatment included sulphonamides or emetine for dysentery or enteritis and mepacrine for malaria in the first 4 days.

We have given nicotinic acid orally and by injection to six cases, four of whom died. It appeared to have no specific effect on the diarrhoea or epithelial lesions in the first 4 days.

There is usually a preliminary fall in weight associated with a diarrhoea as the oedema clears up in the first few days. Once over the critical period the children put on weight rapidly on the diet and gains of more than 1 lb. per week have frequently been recorded. Two cases became grossly obese at the end of convalescence. Exfoliation ceases in 7 to 10 days. The pigment is gradually restored within 4 to 6 weeks. Regeneration of muscle was progressive and complete except for residual flat feet in a few cases. The two cases of prolapsed rectum which survived cleared up without operative interference.

We might summarize our impressions of the response to treatment as follows —

- 1 The effect of riboflavin injections can be noted within the first 2 days on the epithelial lesions of the tongue, lips and external genitals. This effect is also seen on the associated seborrhoea and fissures and on the blepharitis when present. These specific effects have been noted even in cases which did not recover.

- 2 The effect of riboflavin on the fatty liver was not clear-cut. In mild cases the child could consume food containing substances other than riboflavin to which a favourable effect might be attributed. In severe cases the general impression gained was that riboflavin alone was inferior to riboflavin plus liver extract.

- 3 Even though riboflavin deficiency may be the key to kwashiorkor the severe case is probably suffering from many other deficiencies consequent on inanition and these secondary deficiencies as well as the concomitant disease must be taken into account in treatment.

#### NATURE OF KWASHIORKOR.

It is natural to expect that the high incidence of ariboflavinosis in adults would find some expression among the lower age groups. The symptom-complex of kwashiorkor obviously includes at least in a modified form all the adult lesions of ariboflavinosis (see Figs. 1 to 7). The epidemiological

setting and the most outstanding clinical and pathological findings and mode of death point to riboflavin deficiency as the chief factor in causation. Age may account for many of the divergencies between the syndrome of adult ariboflavinosia and kwashiorkor. It is not unusual to find great differences between the adult and infantile clinical pictures of the same nutritional deficiency. Osteomalacia and rickets, adult scurvy and Barlow's disease, adult and infantile beriberi are examples of different expressions of deficiency in youth and adult life respectively. Dr BALDWIN and I (1946) have illustrated the connection between the skin lesions of adult ariboflavinosia and kwashiorkor in a series of photographs at a recent laboratory meeting of the Society. Some of the photographs are reproduced here and it will be noted that the circumscribed lesions of the adult form the nucleus of the widespread dermatitis of the infantile syndrome. I believe that it is riboflavin deficiency which gives to the syndrome its clinical complexion but beyond this it is probably unwise to try to pin down the disease and its mortality to a single cause.' A tropical child suffering from fever, diarrhoea and anorexia for 2 or 3 weeks is unlikely to be deficient in riboflavin alone. It is suffering from inanition, protein deficiency, dehydration and its complex consequences and probably from other vitamin deficiencies.

It is something if we can recognize and follow the thread of riboflavin deficiency through the complicated pattern of kwashiorkor from the social setting of the disease to the stage of fatty liver. It gives us a pointer in prevention. Even if it should be demonstrated that the final hepatic lesion is the consequence of inanition and quite beyond the influence of B<sub>2</sub>-complex therapy it will not alter the position from the preventive point of view. Already many children, showing minimal signs of deficiency not amounting to kwashiorkor, have been treated successfully with food yeast.

#### PREVENTION

Preventive measures must be based on a conception of kwashiorkor within its social setting of poverty, disease and ignorance. As all the indications point towards a primary deficiency of the vitamin B<sub>2</sub>-complex, converging particularly towards riboflavin, the first step in prevention is the supply of cheap sources of the complex in the diet. Milk as a natural source of riboflavin would be the ideal solution of the problem, but milk is expensive. The prevalence of trypanosomiasis rules out the establishment of herds of cattle in Southern Nigeria. Our recent trials with food yeast suggest that signs of riboflavin deficiency could be eliminated in the local adult population with a dosage of 10 grammes daily. The consignment of food yeast which we used was stated to contain 5.4 mg. riboflavin per 100 grammes. The response of our convalescent cases of kwashiorkor to a diet containing this quantity of food yeast suggests that it will be equally effective in children.



## SUMMARY

1 Kwashiorkor is a complex deficiency state in every aspect of which ariboflavinosis is an outstanding feature.

2 The epithelial lesions of kwashiorkor include those of the adult syndrome of ariboflavinosis.

3 The fatty liver and pre-terminal coma of kwashiorkor are characteristic features of experimental riboflavin deficiency in dogs.

4 Signs of ariboflavinosis are seen in 16 per cent. of the adults in the social class from which most cases of kwashiorkor derive.

5 The aetiology of kwashiorkor is complicated by intercurrent disease and the anorexia and inanition which is part of its make-up lead to secondary deficiencies.

6 The complicated and refractory nature of the late stages emphasize the necessity for prevention. The identification of ariboflavinosis at every stage in the aetiology and pathogenesis should bring kwashiorkor within reach of practical prevention by means of food yeast.

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## AMBULATORY TREATMENT OF TROPICAL ULCERS

BY

GEORGE BRECHER, M.D.

*Haiti.*

Numerous methods have been advocated for the treatment of tropical ulcers. STITT'S (1942) textbook mentions twelve different types of local therapy, the more widely used being potassium permanganate baths, neoarsphenamine powder, bismuth paste, excision and skin grafting. Evaluation of reports on these methods is difficult because of wide variation of material and lack of detail. Recent reports by MCGILL (1943) and BEARDSLEY (1943) would indicate however, that excision and skin grafting offer the best chance of rapid cure.

Unfortunately adequate hospital facilities are the exception rather than the rule in many tropical countries where tropical ulcers are one of the major causes of prolonged or permanent disability. Confronted with such a situation on the southern peninsula of Haiti the adoption of a routine treatment avoiding hospitalization and frequent visits to the dispensary was necessary. The only local treatment fulfilling these requirements is the 'occlusive adhesive strip' pressing advocated by THOMAS BAYNTON of Bristol as early as 1799 for treatment of old ulcers of the leg. This method was revived by A. DICKSON WRIGHT (1930) for varicose ulcers of the leg; subsequently it was adopted for the treatment of tropical ulcers by SAYERS (1932) and since then it has usually been combined with neoarsphenamine and bismuth injections (MANSON BAHR, 1941).

### METHOD

- (1) The area surrounding the ulcer is shaved and the skin well dried. Preferably the whole leg below the knee is treated in this fashion. The ulcer is not touched except with sterile gauze to remove excess secretion if necessary.
- (2) Adhesive strips not less than  $\frac{1}{4}$  inch and not more than 1 inch in width are well heated over the flame of a kerosene or gasoline camp stove, to the point of slight blistering

of the adhesive. Each strip is applied immediately after heating in the long axis of the limb, or slightly obliquely but never in a circular fashion. Whenever possible the ulcer margins are drawn toward each other by traction of the adhesive—the end of an adhesive strip is applied to the region above the ulcer and held firmly; traction is applied and the strip then secured below the ulcerated areas. Successive strips overlap slightly and eventually cover the whole ulcer as well as 3 inches of unbroken skin above and below and 1 inch on either side of the ulcer. With moist skins or large ulcers, application of one or more strips in stirrup fashion helps to anchor the rest of the strips. The aid of an assistant is essential to hold the ends of the strips down until all are applied in order to avoid slipping of the strips when traction is applied.

(3) A circular bandage is applied completely covering the length of the strips.

(4) The dressing is changed once a week, or exceptionally after 5 or 6 days in cases of abundant secretion or pain.

(5) Intravenous injections of neosphenamine (maximum dose 0.6 gramme) and intramuscular injections of bismuth in oil (0.13 gramme bismuth subarsenate) are given once a week on the same day. In cases in which treatment has to be continued beyond the 10th week, bismuth only is given from then on.

This method was found applicable to all but two types of cases. Rapidly sloughing ulcers were dressed with sterile gauze and a pad of cotton or cellulose kept moist with a permanganate solution. Injections were given as detailed above, and routine dressings used as soon as the sloughs separated. Ulcers with a considerable amount of luxuriant flesh must first be scraped.

### MATERIAL.

The bulk of our 322 cases answered the textbook description of progressive, sloughing, or torpid ulcers exhibiting little healing tendency. All but two were located below the knee. Their size varied from 2 to 12 cm. in diameter and their duration from 1 week to 12 years. Two-thirds of all ulcer cases were seen in males, although women were slightly more apt to come to the dispensary.

The only diagnostic problem was presented by ulcerative yaws lesions. Yaws is almost universally contracted during early childhood in this part of Haiti. Surveys of certain labour settlements found definite yaws lesions in 100 per cent. of the children under 12. Of our ulcer cases, 44 per cent. showed skin lesions of yaws, 5 per cent. doubtful lesions, 1 per cent. scars of ulcerative lesions. As a group uncomplicated ulcerative yaws lesions differ from tropical ulcers by being frequently multiple, as often located on arms, shoulders, and hips as on the legs, by their rapid response to anti-syphilitic therapy alone and by being as frequent in females as in males. However what appears to be a tropical ulcer may develop from broken-down yaws lesions—transition forms defy classification. Some of our tropical ulcers might have been classified as yaws lesions by other observers, or by more detailed serological and bacteriological study which we were unable to undertake at the time. The majority of our cases, however, seem reasonably well defined by the above-mentioned criteria as belonging to the clinical entity of tropical ulcer either as a complication of yaws or a disease *sui generis*.

### RESULTS.

Altogether 322 cases were treated by the "routine" method described above. 30 of these received food and shelter but remained ambulatory most

of the time. About one third (114 cases) failed to return after one, two or three dressings the average duration of treatment of this group was 10 days. Half of the patients in this group failed to return after a single treatment, indicating the unconcern of the Haitian peasant which was throughout as frequent a cause for failure to continue treatment as was slow progress. These 114 cases are excluded from further consideration because of the short period of observation. Also excluded are three cases with luxuriant granulations which were transferred to a distant hospital for scraping and could not be followed up.

Of the remaining 205 cases —

147 or 71 per cent. were healed.

58, " 29 " " lost track of.

Of the 147 cases healed —

51, or 35 per cent, healed in less than 30 days

74, " 50 " " 30 to 60 days

10, " 7 " " 60 " 90 "

12, " 8 " " 90 " 150 "

The average duration of treatment was 42 days, the longest, 147 days

Of the 58 cases lost track of —

10 or 18 per cent. were almost healed.

36, " 62 " " substantially improved.

12, " 20 " " unimproved when last seen.

The twelve cases that failed to respond to this treatment were 6 per cent. of our group of 205. It is possible, however, that some already improved cases may have failed to go on to a complete healing as we observed two ulcers which broke down completely after initial satisfactory response. We therefore estimate the probable percentage of failures at 10 per cent.

Three of our failures were subsequently healed after receiving the same type of dressing but three mapharsen (0.06 gramme) and one bismuth injection per week. Four visits per week is beyond the realm of possible educational achievement in Haiti and lack of control with such mapharsen dosage makes this method unsuitable for routine use, we believe. It seems worth trying in selected cases, however.

#### COMPLICATIONS

There were two deaths in our series. One patient died 2 weeks after the third, the other two weeks after the sixth, neoarsphenamine injection. These two deaths occurred during the first months of our experience, when we gave only 0.45 gramme doses of neoarsphenamine and no bismuth. The first patient was reported to have refused or vomited all food offered following his last injection. The second developed severe oedema nephritic in distribution. His urine showed only a trace of albumin many leucocytes no red blood corpuscles. Treatment directed toward his toxic nephritis was of no avail.

Since then we have observed a good many cases with anorexia, vomiting, and occasionally with oedema, urinalysis showing essentially the picture described above. Blood pressure remained normal. Hookworm infection—general incidence of which is 80 per cent.—was always present in these cases. Carbon tetrachloride or tetrachlorethylene in cases of oedema, was invariably followed by rapid improvement and neoarsphenamine and bismuth could be resumed with no ill effects. We suspect that neoarsphenamine together with pre-existing hookworm infection is responsible for these symptoms. Such cases as were treated for hookworm prior to administration of neoarsphenamine did not show these complications.

When originally using neoarsphenamine without simultaneous bismuth injections four of our cases developed fresh ulcers below and beyond the adhesive dressing. These ulcers spread rapidly while the original ones often progressed satisfactorily. Substitution of bismuth for neoarsphenamine was followed by arrest of spread and later by complete healing. One of these cases was switched to 3 mapharsen 1 bismuth per week schedule with equally good results. Since giving 1 c.c. of bismuth subsalicylate in oil to every case as outlined above, no such complication was observed.

A slight folliculitis occasionally developing underneath the adhesive strips may be disregarded. It always heals under continued routine treatment as soon as the secretion of the ulcer diminishes.

It is not unusual for ulcers to increase somewhat in size during the first week or so of treatment, but this seems common to all types of treatment (MANSON BAHR, 1941). Occasionally very extensive sloughing begins after treatment is started. This process is always self limited and end results are invariably good, so that we came to consider this type of case as rather favourable. In one case an ulcer progressed slowly but steadily in size from 5 to 7 cm. during a 12-week period (7 weeks neoarsphenamine 5 weeks mapharsen schedule). With no change in treatment, complete healing occurred by the 14th week. This case was one of the three failures subsequently cured with mapharsen.

In three cases the tibia, and in one the calcaneus was involved. One or more sequestra separated completely under routine treatment and could be easily removed with a pair of forceps, revealing sound granulations filling the bony defect. After removal of all sequestrae thus pushed to the surface, further progress was normal.

#### RECURRENCES.

Twelve patients returned with recurrent ulcers. The actual number of recurrences is presumably higher as no other follow up but that provided by patients returning with ulcers to the dispensary was possible. Ulcers over the tendon Achilles and ulcers over osteophytes of the tibia are particularly prone to recur. Half of the recurrences observed belonged to these two types, while

their incidence among all our cases was only 6 per cent. The osteophytes are usually restricted to the area of ulceration, indicating that they represent the end product of periosteal reaction to a deep ulcer

### PREVENTION

Typical tropical ulcers frequently develop from minor cuts and abrasions. The majority of our patients would not come to the dispensary until their wounds had become infected. Since adopting our basic ulcer treatment as a routine procedure for all infected wounds we have not observed any more tropical ulcers developing from such injuries.

### DISCUSSION

THOMAS BAYNTON (1799) considered the greatest merit of the adhesive strip dressing to be reduction of the area to be epithelialized. This is doubtless of advantage whenever the ulcer margins can be drawn together by traction as described above. Frequently however, the ulcer margins are indurated to an extent which precludes an actual reduction of the size of the ulcer though the traction on the adhesive strips may still be of value by diminishing tension on the ulcer edges.

WRIGHT (1930) dealing with the varicose vein complex put the emphasis on the re-establishment of normal venous circulation. Varicose veins however do not appear to play an important role in tropical ulcers judging from our own experience and reports in the literature. Application of the circular bandage from toes to knee, so essential in varicose ulcers did not give superior results to application from ankle to knee only. Moreover even when the bandage was applied from the base of the toes to the knee rest was still beneficial, while varicose ulcer cases may actually be benefited by moderate activity under Unna's paste or elastoplast treatment.

We are therefore inclined to believe that the method succeeds primarily because of adherence to generally accepted principles of wound treatment. Mechanical or chemical trauma at the time of dressing is avoided. reduction of frequency of dressings minimizes risk of superinfection. The sterility of the dressing appears safeguarded by thorough flaming of the adhesive strips.

The rationale of the anti-syphilitic therapy which has been widely adopted was based on the finding of Vincent's organisms in many cases of tropical ulcers even in deeper layers of such ulcers (STRONG 1942). In Haiti such anti syphilitic treatment is especially indicated because of the universality of yaws among children, the doubtfulness of spontaneous biological cure, and the general debilitating influence of the disease.

Our patients subsisted on rice beans mangoes and yams with some breadfruit and bananas—a diet apparently low in higher proteins and calcium. The diet of the 30 patients maintained at the clinic did not differ significantly

from that of the other patients. While the evidence for the role of calcium and vitamin deficiencies in the causation of tropical ulcers is conflicting (CHARTERS, 1943 ORR and GILKS 1931 CLEMENTS 1936) it is probable that better results would have been procured had it been possible to fortify general resistance by improved diet.

### SUMMARY

Results of ambulatory treatment of tropical ulcers with neocarsphenamine and bismuth injections and the occlusive adhesive strip dressing are described. There were twelve failures and twelve recurrences in our series of 20 cases. The method appears valuable because hospitalization can be dispensed with, the number of dressings is minimized, and a large percentage of relatively rapid healing (average duration 42 days) is achieved—three important factors in busy field dispensaries with inadequate hospital facilities.

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## EXCRETION OF STILBAMIDINE.

BY

R. WIEN PH.D (LOND)

*(Biological Division, May & Baker Ltd. London)*

Stilbamidine (4,4-diamidino stilbene) used in the treatment of kala azar is not so readily estimated in body fluids as the sulphonamides, and consequently its distribution in the body is not so well known. A number of investigators have studied the problem, however and shown by fluorescence (HAWKING 1944) and spectrophotometric (FULTON and GOODWIN 1945) methods that the maximum concentration in the plasma of animals after the maximum tolerated dose is about  $40 \mu\text{g}/\text{c.c.}$  falling to 2 and  $0.05 \mu\text{g}/\text{c.c.}$  within 2 to 6 hours. It is thus readily adsorbed but a small concentration is sufficient for a therapeutic effect. The fluorescence method (HENRY and GRINDLEY 1942) has a sensitivity of about 1 in  $5 \times 10^5$  and the spectrophotometric method 1 in  $2 \times 10^5$ . A colorimetric method, based on a reaction with glyoxal in alkaline solution, has also been described (FULLER, 1944) and has a sensitivity of 1 in  $1 \times 10^5$ . Using the fluorescence method KIRK and HENRY (1944) found in kala azar patients that after repeated injections on alternate days of 1 mg/kg about 15 per cent. of the dose was excreted after three injections, and thereafter the rate increased rapidly until about 80 per cent. of the daily dose was excreted, the total excretion eventually reaching about 40 per cent.

In these laboratories we have studied (HAMPTON and MACFARLANE) the urinary excretion in rats employing the fluorescence and glyoxal methods



Since animal urines inhibit the reaction in the latter method a modification was introduced in which the amidine was first extracted from the urine with butanol, and the extract was washed with borate buffer at pH 9.0. The colour reaction was performed in the butanol extract, isopropyl alcohol being added to allow efficient mixing. After acidification the colours were measured photometrically using a green filter having a maximum transmission at about 530 m $\mu$ . A linear relationship was found over the range of concentrations examined.

It was thought of interest, also, to follow the excretion of 2-amino 4,4'-dismidino stilbene, a derivative which is not of therapeutic value but which could be estimated, like sulphonamides (by virtue of the presence of an amino group) by a diazo reaction (BRATTON and MARSHALL, 1939). Since it is also strongly fluorescent comparative results could be obtained by two different methods.

Injections were given subcutaneously once daily for 15 days, and the results are shown in Tables I and II. The doses given were between the

TABLE I  
RESULTS BY FLUORESCENCE AND DIAZOTIZATION METHODS  
(latter figures in brackets).

Number of injections	Percentage excretion of total dose.				
	Amino stilbamidine		Stilbamidine		
	Daily dose mg./kg.				
	1		1	2	3
1	3.2	2.8	0	0	1.4
3	14.0 (32)	14.0	1.5	1.4	1.1
10	27.4 (64)	23.0	8.5	10.3	1.8
15	29.8 (91)	27.4	15.8	14.0	3.3

therapeutic and toxic levels, since it has been shown (WIEN *et al.*, 1944) that repeated injections above 5 mg./kg. in guinea-pigs produce fatty degeneration of the liver.

For amino stilbamidine an obviously greater amount was excreted as determined by the diazotization method than was found by the fluorescence method. The total amino body was estimated, allowance being made for control urines. The results confirmed LARK and HENRY's (1944) finding in that a progressively greater amount of the amidine was excreted on the therapeutic doses (1 and 2 mg./kg.) although the proportion was less in animals

than that found in man. The same was not, however, true at a higher, probably toxic, level of 5 mg/kg where the percentage excreted was much less.

Some of the animals did not survive the total number of injections and allowance was made for this in the figures.

The results in Table II confirmed that with a high dose (10 mg/kg) contrary to what might have been expected, the percentage excretion was very much less than on a low, therapeutic dose (1 mg/kg). The daily amounts excreted on the two doses, however, were similar, the mean figures being  $0.08 \pm 0.007$  mg and  $0.10 \pm 0.01$  mg per rat, a possible explanation being that the kidney could eliminate only a limited amount of amidine. The percentage of the daily dose excreted was (f) 0.05 to 4.6 per cent. (g) 49 to 88

TABLE II.  
RESULTS BY (f) FLUORESCENCE AND (g) GLYOXAL METHODS.

Number of injections	Percentage excretion of total dose.			
	Stilbamidine			
	Daily dose mg/kg			
	1.0		10.0	
	f	g	f	g
5	1.6	41.7	0.4	3.0
10	1.8	51.9	0.5	5.2
15	2.5	58.7	0.6	8.5

per cent. and (f) 0.02 to 1.27 per cent. (g) 5 to 25 per cent. on the two doses respectively, showing a variation which was not large once the maximum rate of excretion was attained. Of equal interest was the fact that considerably more was excreted as determined by the glyoxal than was found by the fluorescence method.

#### SUMMARY

1. There was a marked contrast between a therapeutic dose level (1 mg/kg) and a toxic level (10 mg/kg) in the percentage urinary excretion of stilbamidine in rats.
2. Considerably more (in one series of experiments about twenty times) amidine was determined by the glyoxal than by the fluorescence method.
3. By the glyoxal method the daily excretion reached a maximum after about five daily injections of a therapeutic dose.

4 A large proportion of stilbamidine was presumably metabolized and excreted in a non-fluorescent form.

The preparation of 2-amino stilbamidine is described by ASHLEY and HARRIS (to be published in the *Journal of the Chemical Society*)

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## CORRESPONDENCE

### TYPHUS

To the Editor *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

I have just read with great interest Dr WENTON's Presidential Address on 'Tropical Medicine in War and Peace.' \*

In the section on typhus he mentions attempts at protection in North Africa, Iraq Persia and other places by the use of a concentrated formalinized nickettinal vaccine prepared from egg yolk cultures by the processes of Cox and CRAIGIE. He states he believes the general impression is that some degree of protection has been conferred.

General impressions may be misleading so in 1943-44 there was attempted in Iraq a mass inoculation experiment which it was hoped would settle the question.

Through the generosity of the United States Government, vaccines of the Cox and Craigie types were available in large quantities. The British Army authorities also assisted by kindly allowing certain officers and other ranks to help in the inoculations.

As the typhus season in Iraq is March April, May and June the experiment was begun in December.

A village in the north of Iraq near the Persian frontier, was chosen. Its population was estimated at 3 500 and in the previous typhus season 150 cases of typhus with fifteen deaths had been recorded.

Between 24th December 1943 and 13th January 1944 there were inoculated in the village, with the full course of three inoculations, 1 760 persons with two inoculations only 505 persons, with one inoculation only 2,363 persons.

There was no difficulty in persuading the people of the village to submit to the first inoculation. Indeed, on the 1st day police had to be employed to control the crowd at the hospital. The number of persons who received at least one inoculation, 4 633 was gratifying, probably to be accounted for by under-estimation of the population and an influx on market days.

Second and third inoculations were not so readily accepted and required house to-house visitation and much persuasion. In the last week of inoculation persons who had not previously been inoculated received a double dose.

In the end of June, 1944 after the typhus season, the village was revisited with the object of assessing results.

The general impression of the inhabitants was definitely favourable. On all sides were calls for the syringe, and the children had discovered a

\* WENTON C. M. (1945). *Trans. R. Soc. trop. Med. Hyg.*, 39 3.

new toy a hollow cane with a stick for plunger with which they sucked and squirted water from the ditches. The records showed a lowered incidence of typhus, twenty three cases with four deaths. There were no deaths among those known to have been inoculated, but one patient who had received the full course of three injections developed typhus in a severe form.

Unfortunately for the experiment, but fortunately for the people, the experience of other villages in the neighbourhood was the same. They had, indeed, received some measure of inoculation, but not by any means so much. It had not been possible, nor was it thought desirable, to prevent inoculation, and the energetic Chief Medical Officer of the Lawa used such vaccine as he had with otherwise laudable thoroughness.

Meantime a typhus epidemic broke out in quite another quarter in the Holy Cities of Kerbala and Najaf in the south.

Reports were received from the Chief Medical Officers of several Lawas recording typhus in inoculated persons. There were deaths among them, including that of at least one patient definitely known to have had the full course of three inoculations and at a sufficient time before the beginning of the typhus season for immunity to have been expected to develop.

The appropriate verdict would appear to be "not proven."

Bacteriology Department,

I am, etc.,

Royal Faculty of Medicine

C. P. BEATTIE.

Baghdad, Iraq

## CHOLERA AND ANURIA.

To the Editor *TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene*

SIR,

In my article, *Cholera and Anuria*, which appeared in these *TRANSACTIONS*,\* in quoting CHATTERJEE (page 232) that "Sometimes the cells of the [convoluted] tubules might show degenerative changes," I added in parenthesis 30 per cent. in one series of cases—the proportion doubtless depending inversely on the resistance of the epithelial cells of the tubules to tissue-asphyxiation."

In the light of more recent knowledge, this should have read "the proportion depending on the length of time of survival."

MOON† writes "A serious derangement of renal function‡ accompanies secondary shock [*i.e.*, collapse]. Patients who survive several days in a state of sub-lethal shock [collapse] frequently develop the syndrome of uræmia [anuria] and die of renal dysfunction."

Sydney

I am, etc.,

New South Wales.

J. WALKER TOMES.

\* TOMES, J. W. (1942). *Trans. R. Soc. trop. Med. Hyg.*, 35 229.

† BLOOM, V. H. (1944). *Brit. Med. J.*, 1 773.

‡ Due to anoxia. J. W. T.

TRANSACTIONS  
OF THE  
ROYAL SOCIETY OF TROPICAL MEDICINE  
AND HYGIENE

VOL. XXXIX. No 6 JUNE, 1946

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W ,

on

Thursday, 21st February, 1946, at 8 p.m

THE PRESIDENT

C M WENYON C.M.G. C.B.E. M.B. B.S. B.Sc. F.R.S.

in the Chair

PAPERS

MEDICAL EXPERIENCES OF THE WAR IN THE  
SOUTH-EAST ASIA COMMAND

BY

I—H. L. MARRIOTT M.D., F.R.C.P.,

*Assistant Physician, The Middlesex Hospital, London.*

*(Late Brigadier Consultant Physician, Allied Land Forces South-East Asia Command  
formerly Consultant Physician, India Command.)*

II—IAN G W HILL, C.B.E., M.B., F.R.C.P.E., T.D.,

*Assistant Physician, Royal Infirmary Edinburgh Lecturer in Therapeutics Edinburgh  
University*

*(Late Brigadier Consultant Physician, 14th Army and Allied Land Forces South-East  
Asia Command.)*

III—J. C. HAWKSLEY M.D. F.R.C.P.,

*Assistant Physician, University College Hospital London.*

*(Late Brigadier Consultant Physician Allied Land Forces South-East Asia Command.)*

IV—R. R. BOMFORD M.D. (OXON) F.R.C.P.,

*Assistant Physician London Hospital.*

*(Late Brigadier Consultant Physician, 14th Army)*

## I

Dr H L Marriott I was originally invited to open a discussion on our experiences in S.E.A.C. and it is from this standpoint, rather than from the standpoint of presenting an original contribution that I shall speak. When I left S.E.A.C. I published a paper (1945) dealing with the chief medical problems and lessons as they had appeared to me during the period April, 1942, to April 1945. I have nothing essential to add to what was said in that communication.

During the years of the Burma campaign all of us who were concerned with it learned much. The opportunities for learning were perhaps greatest in the early years when difficulties of every kind beset us and when somehow order and control had to be created out of what was pretty near chaos. This meeting should be of the utmost value if those who learned will speak forthrightly about what they learned. This time we were granted more than 3 years in which slowly and painfully we worked out how to deal with our problems. Next time we may be lucky if we get 3 days. The development of air transport of armies is going to be such that in the next war huge forces may become engaged within a matter of hours in remote tropical areas. The time to apply what we may hear tonight is now. I hope this meeting will not, because we won, be too deeply permeated by an atmosphere of self satisfaction out of which no progress can come.

The primary lesson of all those that we learned was the old one that in warfare in tropical regions medical considerations are of pre-eminent importance and that the medical services can literally play a "match-winning" part. Without good medical work forces will melt away in a few weeks. In 1942 and 1943 units of Eastern Army as it was then called, sometimes had sickness rates of such proportions that practically every man had gone down with sickness and been admitted to hospital inside 2 months. Even in 1944 our hospital admission rate was, in round figures, 1 000 per 1 000 per annum and the ratio of casualties from sickness to casualties from wounds (in spite of much heavy fighting) was 19 : 1. In 1943 the ratio had been 121 : 1.

In my view the most important of the secondary lessons, which should now be applied, are concerned more with the outlook needed in shaping the organization of medical services for tropical war rather than with particular technical advances, important though those advances were. I hope this meeting will not restrict itself to purely technical matters. Technical lessons are most important but they are easily learned and, in any case, new advances will make them out of date pretty soon. It is no good being technically knowledgeable if your organization is such that it does not conform to the realities of tropical warfare and so does not permit full application of the technical knowledge. Changes in organization can only be made slowly—the more far reaching the changes the slower the progress. Anyone who has tried to get alterations

made in the establishments which govern Army medical organization will realize the stability of the institution with which he is dealing. It is essential that it should have stability but the average war does not last long enough to get big changes made. I would repeat *now* is the time to make them.

I would like at the outset to submit as a basis for discussion the broad division into lessons learned about technical matters and lessons learned about organization. I shall briefly deal first with the former and then conclude by dealing with what seemed to me to be the main trends which should govern reorganization of military medical services for tropical warfare.

### TECHNICAL LESSONS

Our chief problems were malaria, diarrhoea and dysentery sprue syndromes, anaemia scrub typhus skin diseases venereal diseases.

*Malaria* was the greatest problem of all and caused about half of all sickness. In its prevention the chief lesson was confirmation of HAMILTON FAIRLEY's epoch-making work (1945) on the efficacy as a suppressive agent of 0.1 grammes of mepacrine taken every day without fail. It caused a remarkable drop in the incidence of sickness from malaria. The value of DDT was becoming demonstrated in the closing stages of the Burma campaign. We also relearned the importance of the old principles of avoidance of native areas at night and of personal protection by nets clothing and repellents. So far as treatment was concerned, our experience demonstrated the value of the 1 week of all mepacrine treatment. It had also the merit of maintaining without break, continuity of mepacrine prophylaxis. We did not learn how to cure relapsing B T malaria but we did learn the proper attitude to inculcate in regard to it.

*Diarrhoea and dysentery* were our second greatest troubles and caused a hospital admission rate of about 100 per 1 000 per annum. The vast majority of minor cases did not get as far as hospital. Few men went without one or more attacks of diarrhoea. Of total hospital admissions, the proportion of cases which showed *Entamoeba histolytica* was about 20 per cent. It seemed that water might be the chief mode of spread for both bacillary and amoebic dysentery. It was noticeable that the incidence of bowel disorders was always highest in the monsoon seasons. Jungle warfare confines men to a few roads or tracks and they perforce defaecate at the sides of the tracks causing very heavy ground contamination. rain then washes organisms into every stream and pool. When on tour of forward areas I made frequent observation and enquiry in regard to water purification and it was obvious that a great deal of unchlorinated water was being drunk. In any case, ordinary chlorination did not kill amoebic cysts. It is essential that some reliable method of water purification shall be found which will kill these cysts. The most useful measure in diminishing the spread of bacillary dysentery was very prompt use of sulphaguanidine which quickly cured men and so prevented them from con



require to be honestly convinced of the vital importance of the measures advocated. The need is to "put over" the simple essential facts to all ranks as vividly as possible by means of lectures, demonstrations, leaflets and films. Unending repetition is essential. We could have taken a lesson from the excellent security propaganda about "careless talk," etc. Now is the time to get inspired education in tropical hygiene established as part of the whole Army's routine training—as important as fighting training. One of the most important sections in any medical directorate should be a section for expert propaganda.

#### FORWARD TREATMENT

It has been pointed out that most of the main diseases can be cured within a matter of days if treated efficiently at their beginnings. The corollary of this is that in tropical warfare treatment should be organized on a forward basis and not on a basis of evacuation of cases to base hospitals.

Malaria can be brought under control in 2 to 3 days and cured within a week. Bacillary dysentery can be cured in a few days. Skin diseases, in most instances, can also be quickly cured if tackled at once. Venereal diseases can be cured by penicillin in 24 hours for gonorrhoea and a week for syphilis. These conditions, taken together, constituted three-quarters of our total hospital admissions. The place to treat them is where they arise or a little in rear of where they arise. In this way not only are men promptly cured but they do not waste days or weeks in travelling; they are not lost to their units and their morale is not sapped by removal from the fighting area. I would submit that for future wars in the tropics we should organize to treat 75 per cent. of medical casualties (I do not refer to surgical cases) in light mobile forward medical units attached to corps or divisions. This was achieved in S.E.A.C. in regard to malaria by the forward malaria treatment units. Provision of base hospital beds, outside the operational area, should not be for more than 25 per cent. of the expected cases of sickness.

If good work is to be done forward, then the pick of medical personnel must be forward—not working in base hospitals. The further forward a medical man is working the more is he putting men quickly back into the fight. The further back he is the more he will be dealing with men who are complete or partial "write-offs" as far as further fighting is concerned. Hence, a large proportion of the best doctors (again I restrict my remarks to physicians) should be working forward.

#### REGIMENTAL MEDICAL OFFICERS

The most forward medical officers are the R.M.O.s and, after hygienists, they are the most important medical officers in an army. A really good R.M.O. inspired with the importance of his function, can do an immense amount in

regard to both prevention and immediate treatment. A dud will just sit about feeling sorry for himself that he is not doing something which he thinks more important, i.e. working in a hospital. The first-class R.M.O. finds it a full-time job and is untiring in maintaining the hygiene and morale of his unit and in watching for and treating at once the beginnings of sickness. It is essential that a tradition should be created that fosters the importance of the R.M.O. A reform basic to such a tradition is that at least half of all R.M.O.s should be majors. As it is, the good R.M.O. is blocked for promotion and driven to aspire to become some sort of 'specialist.' If he is really doing his job he is a specialist—a specialist in man power conservation.

This time last year we were all out there and looked like being there for years. Now we are back and at our old jobs. It might almost have been a dream, but it was no dream. Many of us will often look back on those days as the most significant of our lives and the times in which we learnt most about life. All who knew him well will regret that we have not here tonight Major Gen. TREFFRY O THOMPSON who was D.D.M.S. in Burma during the retreat, then D.D.M.S. of Eastern Army during the hard years, then D.M.S. of 11th Army Group afterwards A.L.F.S.E.A. He was there in defeat and he stayed till victory. Those who had the privilege of working for him consider that the ultimate success of the medical services in S.E.A.C. was largely his triumph—a triumph gained in the face of every kind of difficulty.

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## II

Dr Ian G. W. Hill. As one devoid of training in tropical medicine, apart from the experience gained in war service in the Army, one rises with some diffidence to speak before such an audience in Manson House. It may be, however, that some of the aspects of our work in South-East Asia may prove of interest to the Fellows of the Society and it is in such a spirit that the following remarks are offered for your consideration. No attempt will be made to cover the wide and varied field of disease as met with in that region but two subjects will be selected for comment—

- (i) The trend of sickness rates during the latter part of the Burma campaign, and analysis of the reasons for the low total sickness at this time.
- (ii) Some remarks on scrub typhus met with in localized epidemics, and affecting a considerable proportion of the troops exposed in affected areas.

#### GENERAL TREND OF SICKNESS RATES

In this and the following paragraphs the figures are for the 6 months

October 1944 to March, 1945 covering the period of the advance southwards from the Imphal and Tamu area into the plains of Burma north of the Irrawaddy and beyond. By this time the lessons of previous years had been learnt and applied, and the chaotic conditions quoted by Dr MARRIOTT no longer prevailed. The harvest of reduced sickness then reaped was due to the unremitting efforts of the medical services during the preceding 2 years.

During periods of hard fighting (February-March) medical casualties outnumbered surgical by nearly two to one, while during quiet periods medical cases were anything from twenty to sixty times as numerous as surgical. (See Table I)

TABLE I

NUMBERS AND NATURE OF MEDICAL ADMISSIONS, OCTOBER, 1944 TO MARCH, 1945.

	1944.			1945.		
	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Medical 0.00 per day	3.01	.09	2.18	1.23	1.14	1.12
Surgical 0.00 per day	0.03	0.18	0.10	0.23	0.47	0.41
Ratio Medical/Surgical	90:1	11.6:1	18:1	4.9:1	4:1	1.6:1

In the 6 months October 1944 to March, 1945 the total medical casualties month by month fell by nearly a quarter despite the fact that larger numbers of troops were engaged, and the sick rate per thousand per day fell from over three to approximately one. The last low figure was maintained well on into the hot weather e.g. the total sickness for A.L.F.S.E.A. at the end of May 1945 was 1.19 0.00 per day and in some individual formations it was much lower.

#### ANALYSIS OF RATES OF INCIDENCE OF MAJOR CAUSES OF SICKNESS.

The figures in Table II are derived from returns of admissions to medical units in rear of field ambulances (i.e., casualty clearing stations, forward medical treatment units, hospitals) for the quarter January-March, 1945 in the area of 14th Army and its supporting troops (L. of C. Command). It is seen that malaria, diarrhoea and dysentery and skin diseases together comprise some 45 per cent of all medical admissions to such units. It would be reasonable therefore, to seek in the figures for incidence of these diseases some clue to the fall in incidence of total sickness recorded above.

TABLE II.  
ANALYSIS OF INDIVIDUAL DISEASES. JANUARY TO MARCH, 1943

	14th Army		L. of C. Command.	
	Incidence.	Case mortality	Incidence.	Case mortality
Malaria	25.0 *	0.28 *	23.7	0.21 ~
Cerebral malaria	Very few cases		Occasional cases	
Blackwater fever	Nil		1 case (fatal)	
Diarrhoea and dysentery	7.6 /	—	8.4 %	—
Amoebiasis	—	—	30.5% of all diarrhoeas	
Skin diseases	10.6	—	11.7	—

Accordingly the incidence rates (per 1 000 men per day) for these three types of disease have been analysed in Table III. The incidence of skin diseases shows a fall from 2.03 o/oo per day to 0.87 o/oo per day *i.e.* to two-fifths of the original figure that of malaria from 1.15 o/oo per day to 0.30 o/oo per day *i.e.*, to approximately one quarter and that of diarrhoea and

TABLE III.  
RATES PER 1,000 MEN PER DAY FOR A.L.F.S.E.A. (EXCLUDING CEYLON) FOR THREE  
MAJOR CAUSES OF SICKNESS NOVEMBER 1944-MARCH 1945.

	Week ending					
	12th Nov	30th Nov	31st Dec.	31st Jan.	28th Feb.	31st Mar
Malaria and N.Y.D. fever	1.15	0.75	0.75	0.50	0.36	0.30
Dysentery and diarrhoea	0.18	0.10	0.08	0.07	0.08	0.11
Skin diseases	2.03	1.16	0.71	0.77	0.91	0.87

dysentery from 0.18 to 0.11 o/oo per day *i.e.* a fall of over one third. Among the possible reasons for such reductions in the rates of the prevalent diseases various factors must be considered.

## SKIN DISEASES.

In the case of skin diseases, seasonal and climatic factors undoubtedly played a major role. The ringworm infections, intertrigo, and infected prickly heat cases reached their peak annually toward the end of the rains, and a fall in incidence during the cooler and drier winter months was usual.

## DYSENTERY AND DIARRHOEA.

The diarrhoeal diseases also normally decreased strikingly in prevalence during the winter months, but in the early months of 1945 the expected annual rise with the coming of hotter weather did not materialize. The figure for the end of March was only 0.11 o/oo per day and at the end of May the figure still stood at 0.1 o/oo per day. During the early summer of 1945 the troops were engaged in hard fighting in the plains of central Burma, water supplies were often difficult, and hygiene standards had not appreciably changed from those in preceding years. Yet the incidence of diarrhoeal diseases remained at an unprecedentedly low level. This lessened incidence appears indisputably linked with the use of sulphaguanidine. The drug became available in quantity in September 1944 following the initiative taken by Dr MARRIOTT in calling for several million tablets after his review of the devastating incidence of such diseases in a division after the Imphal battle. In September 1944 the incidence figure for dysentery and diarrhoea was exactly the same as in the corresponding month in 1943. By October a significant fall had occurred, and by November the incidence had dropped to one-half that in November 1943. Throughout the winter 1944-1945 the incidence remained at approximately 50 per cent. of that in the previous winter. The most significant point was that the figures for April, May and June 1945 showed no trace of the great increase usual in these months in previous years, the rate remaining at 0.1 o/oo per day about one fifth of that in the same months of 1944.

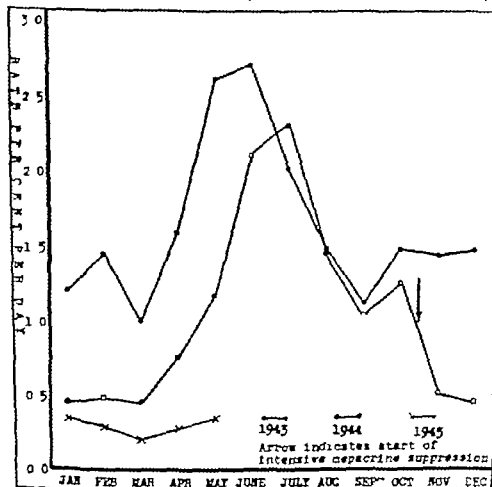
Every effort was made to treat cases of diarrhoea and dysentery as far forward as possible. Unit medical officers were given adequate supplies of the drug and encouraged to use it in full doses in all cases of diarrhoea. The results seemed amply to justify these measures, and are comparable to those quoted by Brig HAMILTON FAIRLEY last year when he reported that in the opinion of many Australian authorities sulphaguanidine saved Moresby. Toxic effects were not seen. The only drawbacks to its use, more theoretical than practical, were (a) that the drug might depress the bio-synthesis of vitamin B in the gut, and (b) that cases of amoebic dysentery might be masked. Against the first objection it may be stated that gross vitamin B deficiencies had been seen in cases of chronic diarrhoea before sulphaguanidine became available for treatment, and were due to malabsorption from the gut, cure or arrest of the diarrhoea is a prerequisite to absorption of the vitamin, whether ingested with the food or synthesized in the bowel. With regard to possible

masking of amoebiasis by the drug it was held that careful sideroom work, with microscopic examination of fresh stools from all diarrhoeal cases in field ambulances and units in rear would prevent undiagnosed cases from returning to duty (Note that during the period March-November 1944 cases of amoebiasis constituted 18.75 per cent. of all dysenteries in 14th Army.)

### MALARIA.

The fall in incidence of cases of malaria was also doubtless influenced to some extent by seasonal factors, but was much greater than had been recorded in preceding years, and as with dysentery the expected rise with the opening

INCIDENCE OF MALARIA 1943-45 (A.L.F.S.E.A. EXCLUDING CYLON)



of the transmission season did not occur. For example at the end of May 1945 the rate was only 0.35 per thousand per day.

Figures of incidence for 1942 are not available, but all the evidence shows that they must have been extremely high. The figures month by month from 1943 to early 1945 when the campaign came to an end, are charted in the above graph. It will be seen that in 1943 the peak was reached in May and June, with rates over 2.5 0.00 per day, while the winter level ranged from

1.0 to 1.5 o/oo per day. In 1944 the winter levels were lower 0.5 in the early months, and again in November and December but in September and October the rate was over 1.0 o/oo per day the summer level was over 2.0 o/oo per day in this year. Throughout the first 5 months of 1945 the rates remained at 0.35 or less. The contrast between April and May of that year and those months of 1943 and 1944 is striking.

The abrupt fall in incidence between October and November 1944, coincided with the enforcement through Staff channels of the lessons learned from the experiments of Brig HAMILTON FAIRLEY and his colleagues at Cairns. At that time senior executive officers accepted the view that malaria prevention through mepacrine suppression was not a medical responsibility but should be enforced by commanding officers, who should be held responsible for high malaria rates in their formations. Mepacrine suppression had nominally been in use for many months, but its rigorous application through non-medical channels coincided with the striking fall in incidence recorded in the graph. As a corollary there was a marked drop in the incidence of cerebral malaria, and in the whole of 14th Army and the L. of C. Command there was only one case of blackwater fever in the 3 months January to March, 1945.

The figures charted are for the A.L.F.S.E.A. forces as a whole. Individual formations showed in several instances curves which bettered those reproduced, and one corps in particular fighting in a highly malarious area in which transmission was proven to be occurring during the spring months, had a total incidence of one half of the A.L.F.S.E.A. figure of 0.35 o/oo per day. Conversely other formations had higher rates. For example, the "A" Indian Division when the incidence for the Army as a whole was 0.35 o/oo per day returned figures of 1.93 o/oo per day one brigade in this division had a rate as high as 15 o/oo per day. The rates for the other two divisions in the corps were 0.24 and 0.30 o/oo per day that for the force excluding the bad division, was 0.27 o/oo per day. Tests of urinary mepacrine levels showed the usual close correlation between high malaria incidence and low urinary excretion of the drug. Action in this case was taken by the army commander following the Australian precedent.

Some of the credit for the decreased relapse rate must be given to the institution of the 7-day Mepacrine-only routine treatment for malaria cases. Under the original regime of 2 days quinine, followed by 5 days of mepacrine, 2 days rest and 5 days pamaquine men frequently circulated for months on end on an endless tour of hospitals, convalescent depots or reinforcement camps and back to hospital. Such men on discharge from a medical unit had had no mepacrine for at least 7 days and had in consequence low mepacrine blood levels. In such a state, relapse might be anticipated in a large proportion. On the introduction of the new routine treatment of 7 days' mepacrine in high dosage (2 days of 0.9 grammes followed by 5 days of 0.3 grammes for British troops) and followed at once by resumption of suppressive dosage of

0.1 gramme per day till discharge, the blood mepacrine level remained high, or if previously low was reinforced and relapses immediately on discharge became unusual. The reduction in man wastage was very marked, and the affected individuals came to realize that their disability had been conquered. There is no doubt that for troops serving in an endemic area attempts to "treat to cure" with combinations of mepacrine and pamaquine are unwise. Hospitalization of individuals is prolonged mepacrine suppression is broken, as shown above, untoward reactions to pamaquine may occur and the men gain a false impression of the seriousness of their infection.

#### INCIDENCE OF SCRUB TYPHUS CHOLERA AND SMALLPOX.

By contrast with the widespread prevalence of the diseases discussed above, other individual diseases played a minor role in the production of casualties. Table IV shows the absolute incidence of three such diseases during the 6 months period already reviewed.

TABLE IV

ABSOLUTE INCIDENCE OF LESS COMMON DISEASES PER MONTH, FOR 14TH ARMY  
(EXCEPT TYPHUS, FOR A.L.F.S.E.A.). OCTOBER 1944-MARCH 1945.

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Scrub typhus	725	678	530	391	40	37
Smallpox	—	—	—	5	10	110
Cholera	—	—	—	About 20 to 30 cases monthly		

#### SMALLPOX AND CHOLERA.

*Smallpox* was encountered as a serious problem among Burmese villagers and refugees at the time of the Irrawaddy crossings and there were fears that spread to our troops might occur. The low incidence which actually occurred is tribute to the efficiency of vaccination among large bodies of men exposed to high risk of infection. So with *cholera* sporadic localized outbreaks of half a dozen cases occurred among the troops without spreading in any instance to assume serious proportions. Immunization against *cholera* was practised but the great emphasis laid on control by hygienic methods without doubt contributed largely if not predominantly to its successful suppression.

#### SCRUB TYPHUS

The die-away of the winter epidemic in 1944-1945 is seen in the figures in Table IV. The disease was met with in localized epidemics limited to the areas occupied by certain formations, and was markedly seasonal (July to January) in incidence. It affected considerable numbers of the men exposed in affected areas and in one corps there were 1 300 cases notified in



the 3 months July to September 1944. Though by comparison with malaria and dysentery the total number of cases was small the disease carried a heavy mortality and was much dreaded by the men.

We were fortunate in that a great deal of careful work on all aspects of the disease was carried out. The clinical features in a large series of 1000 cases have been reported recently by TATTERALL, and several smaller series have also been published. Colonel SAYERS the A.D.P. of 14th Army at the time led a very careful investigation into the epidemiology showing how the disease appeared along the jungle tracks around Imphal and in the valley of the Kaladan. The extreme localization of infected areas was noteworthy. The M.R.C. Scrub Typhus Research Team, with Dr LEWTHWART as its Field Director was responsible for a great deal of detailed work on the epidemiology, vector transmission etc. and close liaison was maintained with the U.S. American Typhus Research Team, led by Colonel MACKIE. Lieut.-Colonel AUDY's work with the M.R.C. Team pin pointing the foci of infection and correlating these with the type of vegetation, has been most interesting and valuable.

With the co-operation of workers in various laboratories from India to Egypt, England and America SAYERS has shown clearly the identity of the disease as met with in this area with tsutsugamushi fever. Blood agglutinations against the various strains of *B. proteus* showed high titres against the OX K strain, agglutination tests with rickettsial antigens showed no significant agglutination with epidemic or murine strains, and complement fixation tests by TOPPING on convalescent sera showed high titres against the Karp antigen.

The fall in incidence in the disease recorded in the early months of 1945 was probably seasonal, though by that time measures of personal protection by dibutyl phthalate were in use and troops were fully "typhus-conscious". A controlled experiment on the efficacy of the Fulton cotton-rat vaccine was planned for the landing operations in the late summer but the explosions of the atomic bombs and the conclusion of the armistice forestalled the projected observations.

### CONCLUSION

It is hoped that this review may yield some idea of the low sickness rates encountered in the latter part of the Burma campaign, and of the reasons for them. To my mind, we were reaping the benefit of lessons learned from the earlier phases of the campaign and of the hard work of the medical services during the preceding years. To mepacrine and sulphaguanidine, freely used, and enforced in the case of mepacrine by executive officers, must go the credit for enabling an army to fight through country notoriously unhealthy with a total sickness rate in the region of 1 per thousand per day.

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## III

Dr J C Hawksley I did not join South-East Asia Command until April, 1945 by which time things were strikingly different from the stages of the war that Dr MARRIOTT has just described. Listening to his remarks brings home the great efforts that had been required to bring the medical services up to their 1945 pitch. The effects upon malaria and scrub typhus of well-controlled suppressive mepacrine and of DBP were specially significant. Whilst diarrhoea and bacillary dysentery were being dealt with more and more by forward units the satisfactory control and treatment of amoebic dysentery was lagging behind—a shortage of EBI was at this stage causing us to rely in many cases upon emetine hydrochloride. The visit of the Consultant in Physical Medicine to the War Office in May enabled discussions to be held upon the need for improving the staffing and equipment of convalescent depots.

In June, after the formation of 12th Army the medical aspects of Burma became largely those applicable to an army of occupation with the exception of the south-eastern parts where the fighting continued. As fresh areas were entered, occasional areas of infestation with scrub typhus continued to be encountered. The scrub typhus vaccine did not arrive in South East Asia until after hostilities had ceased, and the original plan for its controlled use had to be modified—a new scheme (in the hands of Lieut-Colonel CARD R.A.M.C.) will it is hoped, enable some assessment to be made of its efficacy.

The state of the Japanese prisoners taken in July and August by 12th Army illustrated how completely their medical services had broken down. Over 80 per cent. had malarial parasitaemia and diarrhoea was rife. A parachute-drop of medical supplies which the Japanese inadvertently released over our territory might have been expected to contain concentrated supplies of valuable drugs but by far the bulkiest item consisted of bottles of reconstituted Carlsbad salt—presumably all that they could send to their forward troops for the treatment of dysentery. There was a small supply of more useful drugs including a little emetine and some aneurin—the aneurin was in ampoules and proved potent when used to treat cases of beriberi amongst the Burmese in Rangoon.

The suddenness of the armistice in August necessitated quick action in the organization of relief for our prisoners who were scattered over a very wide area, whose numbers were imperfectly known and whose needs in terms of medical supplies were not known at all. The speed of evacuation from Bangkok and Saigon to Rangoon and other similar places made it necessary so to divide up the available essentials for treatment that adequate quantities could be dropped or landed in places still in Japanese hands whilst the big receiving areas were given an adequacy. The principal anxiety was lest valuable stores were over-supplied by air to places from whence they could not be got back if more urgently needed elsewhere a few days later. The medical officers

The main deficiency diseases seen during the occupation were —

Beriberi. Cardiac and neuritic.  
Glossitis and angular stomatitis.  
"Granular cornea" and interstitial keratitis.  
Retrobulbar neuritis.  
Sore scrotum.  
Eighth nerve deafness  
Painful foot syndrome  
Spastic paraplegia.  
Encephalopathy  
Pellagroid skin eruptions.  
Low plasma-protein oedema.  
Malnutrition diarrhoea.  
Anaemia.

All attributed to deficiency is  
Vitamin B complex

The association of retrobulbar neuritis, 8th nerve deafness and spastic paraplegia, has recently been regarded as a syndrome but one questions whether it was anything more than a coincidence.

Of other diseases malaria was not as serious a problem on Singapore Island as it was in Siam. dysentery especially amoebic dysentery on account of the extreme scarcity of emetine caused much illness and diphtheria, more than half the cases of the cutaneous variety in some outbreaks, was a serious problem. Cutaneous diphtheria was often due to a secondary infection of the "sore scrotum" mentioned above.

The prisoners evacuated through medical channels, as we saw them in September 1945 fell into two classes—the great majority who were fit for evacuation by hospital ship and a minority who were seriously ill. One must remember that most had already been on a better diet for several weeks when we saw them. The majority were severely wasted, with sallow complexions and sunken eyes. Most of them had some weakness of the legs often with absent tendon jerks, and many had swelling of the legs and ankles, apparently due to low plasma protein oedema rather than to beriberi. Most had numerous scars on their legs, and many had septic sores or frank tropical ulcers. Roughness of the lips and some scarring at the angles of the mouth was almost universal. A large number had some degree of diarrhoea, and a small proportion had visual defects of varying severity following retrobulbar neuritis.

Amongst the severely ill patients a few had frank cardiac beriberi, with acute heart failure. Some of them died and some recovered. They were treated with large doses of thiamine intravenously and in some cases with digoxin in full doses. A larger group suffered from gross wasting "malnutrition diarrhoea," and usually some oedema. We soon learnt the dangers of any form of transfusion, or of any incautious additions to the diet in these cases. It was difficult to make some of them take any food at all by mouth, and the starvation appeared to have reached an irreversible stage, but some of them, who were persuaded to drink three or four bottles a day of a protein hydrolysate

of Indian manufacture—something of a feat, for the preparation was very unpalatable—improved quite rapidly. The last group of severely ill patients consisted of Indians with advanced pulmonary tuberculosis, many of them dying. For them there was evidently little or nothing to be done, beyond measures to make them as comfortable as possible.

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## DISCUSSION

Lieut-General Sir Alexander Hood\* I came here tonight to listen. I did not come prepared to speak, but I am very glad to have this chance of paying a tribute to the consultant physicians whom you have heard here. They did a really first-class job of work under the most difficult conditions. They were working with shortages of personnel, shortages of drugs, shortages of transport and under conditions which had to be seen to be believed. You will understand this when I tell you that in order to make a road in some parts of Burma you had to import the stone from overseas mostly and there was probably only one month of the year when you could make the road. In a jeep it took me once  $3\frac{1}{4}$  hours to do 8 miles, so you will understand some of the difficulties they were up against. Brigadier MARRIOTT gave us some ideas about organization and I would like to comment on that. First of all we were not equipped or prepared either with men or with materials to fight more than one war at a time, and we had to give priority to the theatre which everybody thought most important at any particular given moment. It was first of all the Middle East when we had to put everything we had in the way of personnel and equipment into Montgomery's 8th Army. Then it became North Africa and Italy and we gave them everything. Later it was North-West Europe, and lastly came the 14th Army which had been waiting all that time to become first priority. The time came when we were able to give everything required, and you see by the fall in the incidence of disease that they got all they required and they made excellent use of it. Brigadier MARRIOTT made a point of the importance of hygiene officers. I yield to none in my admiration of their work, and the Army is indebted to them for all they have done but what Dr HILL said, and the chart he showed indicates that what is required is to inculcate into the Commanders in the field the necessity of hygiene measures before they can be carried out. That was our greatest difficulty and while Brigadier HAMILTON FAIRLEY's work in Australia was a magnificent effort, even greater was the fact that he was able to convince the Commanders of the Australian Forces. It was not until he got

\* Director General Army Medical Services.

it put over to the Australian staff that it became a court martial offence for a commanding officer to have more than a certain number of malaria cases in his unit, and that if the figure was above that he would be automatically removed, that the rate fell. We knew mepacrine would give good results. We had tried propaganda of all sorts with no great success, but when Brigadier HAMILTON FAIRLEY got it over with the Command of the Australian Army we got results. When I went out at the end of 1944 and stayed with Corps Commanders, I was astounded at the knowledge they had of malaria, mepacrine levels in the blood, excretion in the urine and everything else. They put it across and there was a terrific fall in the incidence of malaria. Before the Supreme Commander in Chief South East Asia Command, went out to his Command the three Director Generals of the fighting services met him and talked to him round the table. We told him that he was not going to fight the Japs but malaria and other tropical diseases, and that his Chief Staff Officer was his Director of Medical Service. He was very much impressed. I was very much delighted to hear the tribute paid to our old friend General TREFFRY O. THOMPSON. I saw him at the end of 1942 when he had just come out of Burma after the retreat which was one of the greatest disasters that befell any of our troops in the whole war. I saw him again at the end of 1943 and went all over Burma with him. I agree with Brigadier MARRIOTT that no man did more for the British Army than General THOMPSON.

Colonel J. Bennet. I was just thinking of the enormous complexity of this subject. An indication of this is given in the table one of the speakers has shown on the screen. In some cases a single item from the conditions listed would provide material for considerable discussion, and I would suggest that this be deferred until an occasion providing adequate time for a full review is presented. I would go further and say that some of these conditions, such as protein deficiency oedema, merit an evening's discussion devoted solely to their elucidation. Critical levels of food factors in the diet have to be studied in relation to existing conditions. Amongst these the compulsion to hard work seems to have been one of the most potent factors in producing the profound state of ill health reported in some of the groups. But all require study in the light of the total existing situation, diet, co-existing disease and compulsion to hard labour and I feel that one could do no more than scratch any of the problems by raising them now. I suggest that the matter should be subdivided and dealt with in parts, a full evening's discussion being devoted to each.

Dr E. A. Bennet. One topic which has not been referred to in the paper or discussion, namely the incidence of psychiatric disorder was a source of great difficulty in the early stages of the campaign in Burma and a cause of numerous casualties. Many patients thought to be suffering from nervous

or psychosis because they presented psychiatric symptoms were, on investigation, found to be suffering from malaria, or that malaria and/or some other condition was a complicating factor in a psychiatric illness. This is of importance in a survey of illness in the tropics.

We made it a rule—I think it important—that every patient presenting psychiatric symptoms must have a physical examination and that the blood must be examined for malarial parasites. We had difficulty in enforcing this and in certain areas an order was issued that the medical officer must initial a statement that these examinations had been carried out. There was a tendency—not restricted to the tropics—to forget that a patient with psychiatric symptoms might have a physical illness if a patient exhibited psychiatric symptoms (particularly of conduct). Some M.O.s seemed to think that neurotic symptoms always stand alone, and that no investigation is needed. Mixed conditions were the rule rather than the exception.

Dr MARRIOTT referred to organization. The campaign in Burma was conducted in difficult country, and this necessitated a special psychiatric organization which was unique in the British Army—each division was provided with a small psychiatric unit consisting of a psychiatrist, one or two specially trained orderlies and in certain divisions a motor truck. A supply of drugs and other equipment, suitably packed, was provided. This provision of Divisional Psychiatrists worked well in practice. It is worthy of close consideration in the medical planning of a campaign in tropical countries—or indeed in any country. The psychiatric casualties in Burma were very high in 1942 and in 1943. In 1942 there were no psychiatrists working on the Indo-Burma front. In setting up an organization for this campaign and for the troops in India, we had, therefore, the advantages and disadvantages of starting from scratch. In 1943 and 1944 the psychiatric casualties were dealt with far forward and their number was much reduced. From first to last we received the full support of the DMS in India and of Major-General TREFRY O THOMPSON who was successively DDMS of Eastern Army 11th Army Group and finally DMS of A.L.F.S.E.A. The organization described had also the support of Corps and Divisional Commanders who were in a position to estimate the benefits resulting from it, particularly in the saving of man-power.

Sir Phillip Manson-Bahr said that he had examined a good many ex internees from Eastern prison camps. Most had suffered from oedema of the legs and abdominal wall but he was struck by the fact that those who came from the British Zone were diagnosed as wet beriberi whilst those from the Dutch East Indies were labelled hunger oedema.

It seemed to him that they were all suffering from the same deficiency and that the true diagnosis was in fact the latter. If beriberi why was it that recovery was so rapid without residual nerve involvement?

**Dr R B MacGregor** As one of the prisoners whom Dr BOARFORD talked about, and one who has suffered to a slight extent, and observed to a bigger extent, these cases of beriberi and hunger oedema, I suppose the only reason why the British think of the condition as beriberi is that it is easier to say than hunger oedema. We had both sometimes occurring at the same time, sometimes separately sometimes in different individuals, or in the same individuals at different times. The question of oedema alone as we saw it in prisoners and civil internees, would take up all the evening, and I would not attempt to discuss it now. One generalization I would like to make at this stage is that in our civilian internee camp we had an almost completely controlled experiment in the deficiency diseases. We had people living precisely the same life, on precisely the same diet, down almost to the last gramme, and the most striking feature was that the men's reactions were so different. At one time you had two or three individuals out of one hundred affected and the remainder apparently well. If you had one individual with a beriberi complex, another might be suffering from defective vision, while the remaining ninety-eight individuals out of the 100 at the same period would be apparently well—there never seemed to be any reason why there should be such very pronounced idiosyncrasias. Of all the generalizations that was the most striking the individual differences in people living under precisely the same conditions. Another general observation which I have noted before was that during the period when we were all suffering from shortage of calories, or lack of carbohydrates, the people who suffered most were the older men almost in direct proportion to age was the loss of weight that was suffered on a diet deficient in carbohydrates.

There is another form of oedema which was very noticeable and surprising, and that was not the oedema of hunger but the "oedema of plenty" which occurred to a very great extent in some people and to a minor extent among certain others. When we were homeward bound on board ship, being well fed, comfortable and under for us, ideal conditions, this oedema appeared in people who had not had the slightest traces during internment. It disappeared spontaneously after a week or two or sometimes a month or two.

**Dr F Murgatroyd** The incidence of the "oedema of plenty" as it has been called was remarkable, and many prisoners who had been subjected to prolonged malnutrition became oedematous when their diets were increased.

Among undernourished persons oedema may also be found without grossly obvious cardiac or neuritic signs and without low plasma protein levels. The exact mechanism of production of this oedema does not seem to have been clearly explained. It may be associated with increased capillary permeability due to malnutrition or as blood pressures are usually low in persons suffering from chronic starvation with diminished renal filtration and inadequate elimination of fluid. This latter may possibly be reflected in, and partially

compensated by the nocturia which often preceding manifest oedema, was experienced by so many under-nourished persons not only in the prison camps of the Far East but also among the semi starved populations of Europe. On the other hand nocturia may represent a failure of under-nourished renal tubules to reabsorb water adequately.

**General A G Biggam** I would like to say how heartily I agree with the speakers who have emphasized the importance of preventive medicine in tropical combat areas. When one considers the state of our knowledge of protective measures against malaria and scrub typhus now compared with the early days of the war one realizes what enormous strides have been made in personal protection against these diseases.

In Burma before the Japanese invasion, our troops training in the jungle even under peace-time conditions, suffered severely from both malaria and scrub typhus. We have heard from the speakers this evening how the whole picture has changed and how even when in close contact with the enemy our troops can now be provided with means of protection which, if properly applied, will keep them fighting fit.

**Brigadier MARRIOTT** has pointed out the high incidence of amoebic dysentery amongst our forces in Burma and Assam. That this disease was prevalent in Burma had been recognized for a long time, the reason for this is not quite clear but one possible cause is contamination of uncooked green vegetables by the Chinese market gardeners whom we know very frequently employ infected material for fertilizing these crops. I agree we have not yet found an entirely satisfactory method of treating amoebic dysentery but I can say without any hesitation that the whole outlook for the patient returned to this country suffering from very chronic amoebic dysentery has been entirely changed by treatment with penicillin and sulphonamide prior to the administration of the specific anti amoebic drug. It is seldom now that we fail to cure such a patient even when his history is one of very long standing disease which has failed to respond to many courses of treatment.

I would like to add my thanks to the speakers for the excellent papers they have read.

**Dr L E Napier** I must apologize for introducing a new note into the discussion, but I cannot help admitting that I was horrified to hear that it was the end of 1944 and the beginning of 1945 before principles of malaria prevention that had been laid down 10 years before were applied effectively. I am referring to the use of prophylactic atabrin. **Sir ALEXANDER HOOD** has explained this and I think that his remarks about **Dr HAMILTON FAIRLEY**'s work were very true namely that its greatest value lay in the selling of the idea of prophylactic atabrin to the combatant personnel. The dosage that was used in the pre war days was admittedly too small but it should not



*Terebra* and the Turridae have apparently never caused human accidents, but at least five species of *Conus* (Plate I) have been, admittedly on rare occasions, responsible for serious and even fatal accidents to human beings. They are not used as food as far as I have been able to ascertain but they are sometimes used as fishing bait and their beautifully camouflaged shells are very attractive to conchologists so that the accidents reported have all occurred among fishermen and shell collectors.

From 1848 (when the first case was reported by A. ADAMS) until 1932, all such accidents were confined to the Western Pacific, although the species of *Conus* responsible are widely distributed in the Indo-Pacific region.

It is the purpose of this article to relate the facts of an accident which came to my clinical notice in 1932 while in practice in the Seychelles Islands, Indian Ocean, and describe some original work I have since done in connection with two species of *Conus* from Seychelles in an attempt to solve several problems about which available medical authors were silent.

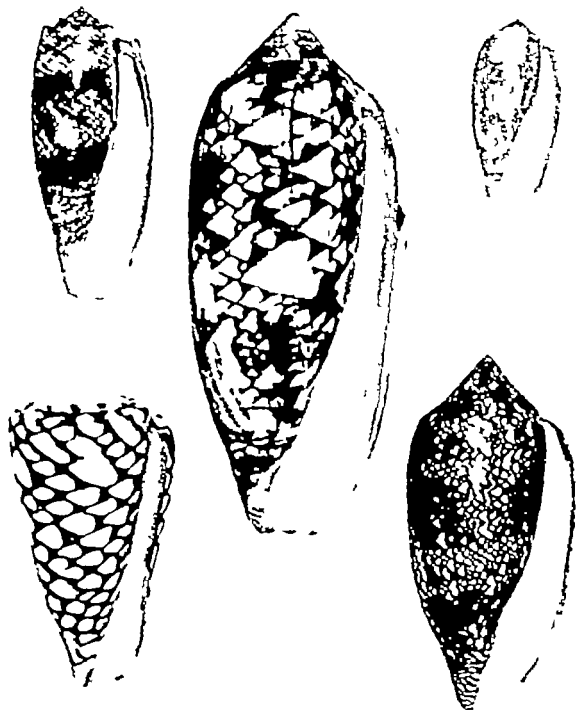
Owing to the extreme difficulty I have experienced in following up the original accounts of human accidents from various species of *Conus* because such accounts appear in widely different publications, private reports and museum records, extending over nearly a century (1848-1943), I have considered it useful for future workers in this field to reproduce in full in Appendix II the pertinent sections of all those I have been able to trace either in the original publications or quoted in articles by later writers who had access to the original versions.

The reasons for the delay in publishing this article will be apparent in the course of the article.

#### HISTORY OF THE CASE.

In June, 1932, a white Seychellois of French descent, Monsieur FRÉDÉRIQUE DE LAFONTAINE, 32 years of age, residing at Ile-aux Cerfs, Seychelles (Plate II), was wading at low tide in the shallow water of the lagoon not far from the shore of the island, in search of shell fish, when he found and picked up a medium-sized living male specimen of *Conus geographus*.

As the shell of the mollusc was covered with a slimy growth of marine algae, he proceeded to clean it up by holding the shell in his left hand, aperture towards the palm, and scraping the shell with a pocket knife. He had hardly started scraping when he felt a sharp sting in the palm of his left hand, followed immediately by a burning sensation. Turning the shell over quickly to look at the part of the animal visible in the aperture he saw the "mouth" of the animal just retracting into the shell, and noticed at the same time what he described as a "fine sharp needle protruding from a narrow tongue-like organ which was gradually withdrawn out of sight into the mouth before the latter had completely retracted into the shell. When once the animal had



4

3

5

The five species of *Conus* so far reported as having stung human beings

- 1 *Conus geographus* 2 *Conus tulipa* 3 *Conus aulicus* 4 *Conus matris* 5 *Conus textile*

The coloured patterns on the exterior of all these shells vary from orange to dark brown on a pinkish white background. The internal surface visible in the aperture is pale bluish pink.



1. Longitudinal section of distal end of proboscis and prephary with three muscular ocella (H & E) (x 30).



2. Transverse section of proboscis with tooth in prephary distal to muscular ocellar (H & E) (x 30).



3. Transverse section of proboscis with tooth ligament in prephary proximal to muscular ocellar (H & E) (x 30).



4. Transverse section of highly epithelialized tubular poison gland (H & E) (x 30).



5. Radial sac dissected to show the teeth in the tooth arms anchored by ligaments to the wall of the sac (x 3).



6. Transverse section of long arm of radial sac showing the teeth in two rows (0° and 180°) at different levels. Ligaments are also seen in section (from Haemulon 1) (x 30).



Longitudinal section of Conus prephary showing radial sac (a), tubular gland (b) and poison sac (c) in transverse section. (x 30).

completely retracted itself into the shell, neither the needle nor the tongue-like organ were any more to be seen.

The wound itself was so tiny as to be almost invisible. The burning sensation soon gave rise to numbness, and within a few minutes he felt his left arm tingling and gradually becoming numb. Then feeling his head getting "queer" he wisely decided to regain the shore and go home. I have used the word "wisely" because within an hour the whole of his body was numb, his sight was impaired, he had marked dizziness and nausea, he became completely paralysed and could not move his limbs or sit up and speech was difficult. Had this occurred while he was still in the water he might well have lost his life by drowning.

After 5 or 6 hours he began to improve a little and asked to be taken to my surgery at Mont Fleuri on the main island of Mahé 3 miles distant across the lagoon (Plate II, p. 488).

The accident had occurred at 9 a.m. and he was brought by pirogue (canoe) to my surgery at 6 p.m. having been carried to and from the boat in a long chair. He was still giddy and unable to stand, with a feeling of general weakness in all four limbs.

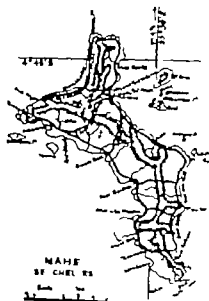
On examination there was nothing to see at the site of the sting and there were no signs of hypersensitiveness such as urticaria. His knee reflexes could not be elicited but his pupils reacted normally to light and to accommodation. The pulse rate and respiration were normal. There had been no respiratory difficulty at any time or any rise of temperature as far as he could tell. When examined his temperature was normal.

These were obviously symptoms of some form of neuro-toxic poisoning and the only treatment I considered might be of any use was after a hyperdermic injection of strychnine hydrochloride (1/60 grain), to send him home to bed with a strychnine mixture and recommendation that he should be given general massage in addition. Possibly this was unnecessary but it was carried out on his return to Ile aux-Cerfs and, in spite of it, it took 3 days for the weakness to disappear completely.

When he was brought to my surgery on the day of the accident he had, fortunately ordered the mollusc also to be brought alive in a vessel containing fresh sea water and sand, and as I was at the time indulging, in my spare time, in the fascinating hobby of collecting and classifying the marine shells of Seychelles the species was easily diagnosed as *Conus* (*Gastrium*) *geographus* Linn. of the empty shells of which I had several specimens in my collection (Plates I and III). It was not a large specimen, measuring only 8.5 cm. in length.

Knowing nothing about the mollusc beyond the characters of the empty shell I at first found it difficult to believe that such a small animal could have produced, at one short sting such profound general symptoms in an adult man. However knowing the patient to be honest and intelligent, I decided

## PLATE II.



Ile-aux-Cerfs from Victoria, Malé. Port Office in foreground.



North west corner of Ile-aux-Cerfs. Note local canoes (pirogues) and Malé in the background (3 miles distant)



Northern half of Anse Boileau Bay on the West Coast of Malé, a fertile source of *Conus geographus* (x).

to dissect the animal and at least check his statements regarding the needle-like weapon he had observed and endeavour to locate the origin of a possible poison responsible for the symptoms the patient had displayed.

I had no difficulty in finding the sharp needle (later ascertained to be a radular tooth) fixed within and protruding from the end of the tongue like organ (later ascertained to be the proboscis) inside the hollow cylindrical mouth (later ascertained to be the rostrum). This was indeed fascinating and I immediately began to search available literature on the Mollusca for further information.

The only works on Mollusca available on the island gave me practically no information on anatomy and none on the radula.

At that time Mr R. C. WOOD who was spending a few months in Seychelles and resided at Ile-aux Cerfs for the purpose of collecting specimens of fish and molluscs, hearing of my difficulty very kindly lent me Volume 3 on Mollusca by A. H. COOKE, *Cambridge Natural History* (1905) and on 23rd June 1932, wrote to me as follows —

(1) The *Royal Natural History* (1894) has the following remarks *re* Conidae —

"The Radula is composed of two rows of long barbed marginal teeth. Some instances are on record of persons having been bitten by cones when handling them and it is said that the bite to some extent, is poisonous, but whether this character is peculiar to a few or common to all species, we have no means of knowing.

"(2) Ex. article by G. C. ROMOV in *Animals of All Countries* (1924) —

"Number of teeth on radula varies from sixteen to 750 000 in different classes of molluscs.

The Conidae, Terebridae and Pleurotomidae (now named Turridae) constitute a separate group of Stenoglossa known as Toxoglossa. The possession of a "poison gland," of a radula with only two teeth in a row and the absence of jaws, are their distinguishing traits.

"The radular teeth are large and often strongly serrated, being quite formidable looking structures. In addition, these Gastropods are peculiar in possessing a special gland opening into the middle of the oesophagus known as the gland of Leiblin. This structure in the group Toxoglossa has an irritating secretion which may be regarded as poisonous, for although a bite from such animals is fatal only in exceptional cases it often causes severe illness."

With the above information, particularly from Cooke's *Mollusca*, I proceeded to dissect further specimens of *Conus geographus* and acquired a clear understanding of the gross anatomy of the poison apparatus, but not of the actual source or nature of the poison or of the mechanism by which it is introduced into the victim.

The specimens were obtained from Cerf Island and from the southern part of Anse Boileau Bay on the West Coast of Mahé (Plate II).

Some species of cones have been collected at great depths by expeditions but the ordinary collector confines himself to the shallow waters of lagoons and reefs. In the Seychelles most of the islands including the main one Mahé, have an irregular festooned outline forming small concave bays. Between the land points that jut out to give the bay its concavity a line of coral reefs usually fairly straight converts the water on the land side into a lagoon of

varying depths (averaging about 6 feet) from the deep sea outside it. At low tide the water recedes from the lagoon leaving the coral reefs and extensive fields of seaweed exposed, except for some clear sandy pools or pools containing growths of coral and sea-weed of various species. In the latter pools *Conus geographus* could be found usually in the neighbourhood of small localized coral growths. They usually go about in pairs on the sandy bottom among the weeds but occasionally as many as eight could be found in a small area (Anse Boileau Bay Plate II). I was not successful, however in obtaining any from Anse aux Pins Bay (south-east coast) although they must be at home there.

On leaving Seychelles a few months later (March, 1933), I brought with me many specimens of zoological interest which I presented to the late Professor ASHWORTH at the Zoological Department of Edinburgh University including some specimens of *Conus geographus* and *Conus millepunctatus*\* on which I intended to do some further work for publication. This I carried out later partly at the Royal Infirmary Sheffield, and partly (animal experiments) at the University of Sheffield. During this work a search of literature revealed that similar work had been done on *Conus tulipa* and *Conus textile* by H. O. SHAW (1914) and on *Conus mediterraneus* by ALPERS (1932). My anatomical findings in connection with *C. geographus* were incorporated in a paper which I read before the Medical Sciences Club of Sheffield University on the 9th May 1936.

I felt, however that the paper was not worth publishing until I had done some further work to elucidate the nature of the poison, but for this purpose it would be necessary to obtain some material. At the above meeting it was suggested by Dr E. T. B. FRANCIS, of the Zoological Department of the Sheffield University that the best way of obtaining poison would be to have it extracted from the crushed poison apparatus in 30 per cent. alcohol.

I accordingly worked out carefully the steps of dissection of the animal with clear illustrations of each step (partly illustrated on Plate II) which I sent, along with labelled bottles, dissecting instruments, mortar and pestle, sand, reagents etc., to Mr JOHN TYSON of the Cable and Wireless Station in Mahé, who was to get in touch with Mademoiselle AGNES DE LAFONTAINE, my ex patient's sister at Ile-aux-Cerfs, who I knew was interested in shells. I had every confidence that with her brother's help she would be able to supply my requirements.

Except for having to wait many months I was not disappointed, for eventually I received from her further whole specimens of *C. geographus* and *C. millepunctatus* (which I had also asked for because of its much larger average size than that of the former) and collections of the isolated poison apparatus of

\* I have used the familiar name *Conus millepunctatus* Lamarck, but this name has unfortunately been used earlier for a different species and by the strict rule of priority it should be called *C. leopardus* (Röding).

both species crushed with sand in 30 per cent. alcohol. From a second lot of each, the poison was obtained by syringing out the poison gland and duct into a clean sterilized receptacle with 30 per cent. alcohol. A third lot consisted of a collection of the poison apparatus removed from the animals and placed in 30 per cent. alcohol.

On receipt of these I proceeded to carry out some experimental work on mice. The extracts from the three lots were carefully filtered and evaporated in a water bath until reduced to brown oily liquids and dilutions of various strengths made in sterile distilled water for injecting into mice.

I was now to be disappointed, however for the material proved completely inactive both in the case of *C. geographus* and in that of *C. millepunctatus*, even in the undiluted form, and whether injected intraperitoneally or intramuscularly. I have accordingly considered it unnecessary to publish the protocols of my experiments. This part of the work remains to be carried out afresh and there seems no other way out of the impasse but that this part of the investigation should be carried out in the tropics where the poison could be obtained fresh from the living animal. The prospects of my doing so are very remote. Perhaps these notes may stimulate some worker in the tropics to undertake the work and at least afford anyone interested a rapid and easy guide to the anatomical structure of these animals knowledge of which is essential for the successful extraction of the poison.

Apart from its dangerous property it is possible that the poison of *Conus* may be of use in medicine, just as viperine venom has proved to be, although in a different capacity. In this respect it is interesting to note that the effect of *Conus* poison (paralysis probably of centres of voluntary movement) appears to be the direct opposite of that caused by the poison of stinging fishes of the genus *Synanceia*, which produces convulsions or toxic spasmodic symptoms akin to tetanus. *Conus geographus* and indeed many other species of *Conus* occur in similar ecological localities in these tropical island lagoons as do species of *Synanceia*. These venomous fishes when conscious of danger usually bury themselves in sand with the venomous spine of their dorsal fin erect and protruded, and accidents occur when wading barefooted individuals tread on them unawares. In Seychelles accidents are not infrequent from this source among fishermen wading at low tide after octopus and lobsters or in search of *Pinna muricata* (locally known as *ache d'arme*) which is widely sought after as bait for putting in *caniers* (local name for fish traps). Locally species of *Synanceia* are known as *laffe* and are not infrequently caught in fish traps along with other fish, and accidents may occur if they are carelessly handled.

The apparent inactivity of the alcoholic extract I used in my experiments may have been due to one or to a combination of factors such as the necessarily prolonged storage in alcohol in the warm tropical climate, or the fact that the mice I used may have been refractory to the poison (if still active).

According to RAPHAEL DUBOIS 1903 (quoted by CALMETTE, 1908) while



sea and fresh water fishes (golden carp) are very sensitive to the poison from the purple gland of *Murex* warm blooded animals are refractory. The effect of the latter poison on man is not stated and is probably unknown. That *Conus* poison produces severe effects in man there can be no doubt. Whether the inactivity of the extracts of *C. geographus* and *C. millepunctatus* poison I used was due to refractoriness on the part of the mice, or was the result of some oxidizing process during prolonged storage in alcohol in the tropics, cannot at this stage be determined. Unfortunately when I carried out my experiments, I was not aware of DUBOIS' observations on *Murex* poison and I did not ascertain the effects of my extracts on frogs or fish.

DUBOIS extracted the poison of *Murex* by crushing the purple glands with sand and alcohol (strength not stated by CALMETTE). The alcoholic liquid was filtered and evaporated in a waterbath and yielded a brown oily liquid, subcutaneous injection of a few drops of which into a frog was sufficient to produce very toxic effects. Sluggishness and slowness of movement were seen to supervene fairly quickly. Reflex actions were still exhibited, but the animal was no longer able to jump. If the dose be not too strong, this condition of paresis lasts for several hours and then disappears. In most cases, however, the paresis is succeeded by complete paralysis, and the animal appears as though suffering from curare poisoning. Yet, writes CALMETTE, the fact is that the venom is neither curare-like nor cardiac—the heart, muscles and motor end plates, and motor and sensory nerves are spared—the nervous centres alone are attacked, especially the encephalon. The animal dies without convulsions.

The above description of the effects of *Murex* poison on the frog would appear to be very similar to those of *Conus* poison on man, on the segmented sea-worm *Nereis* (ALPERS, 1932) and on the octopus (BRUCE CUMMINGS 1936).

The only attempt to investigate the source and nature of the poison of *Conus* as far as I have been able to ascertain from literature, is that of ALPERS (1932) in connection with *Conus mediterraneus* Linn. the only species found in European waters and limited in distribution to the borders of the Mediterranean Sea and west coast of Spain. His observations were carried out at the Naples Zoological Station.

After observing repeatedly that whenever *C. mediterraneus* moving with its rostrum and siphon protruded, met with the segmented marine worm *Nereis* the latter went into peculiar spasmodic convulsions and made vigorous writhing movements to escape. The head and tail segments assumed a striking stiff attitude and bent with a slight upward curve while the head became much swollen. If the prey was thus exposed several times the symptoms increased in intensity leading eventually to complete paralysis after which it was easier for the cone to "swallow" the victim. (Apparently *Nereis* is one of the marine animals on which *C. mediterraneus* feeds.)

Believing that the victim's behaviour could only be explained by the action of the secretion of the poison gland, ALPERS decided to study the effect of its secretion by isolating the poison "gland" and duct from a living *Conus* and squeezing the secretion into the water close to a *Nereis*.

His experiments failed in their purpose, evidently because he had not realized that the substance must be injected into the tissues of the victim. He

had based his experiments on the single observation that when cracking the shell of a living *Conus* for the purpose of determining the position and state of a swallowed *Nereis* the cone had squirted a thin jet of watery clear liquid from its fully retracted rostrum. This he believed might be the poison from the poison apparatus, whereas it was most probably only sea-water. He never succeeded in getting other specimens to repeat the squirting and also failed to make the animal sting his finger.

The anatomical arrangement is such that the poison can only be injected through a radular tooth firmly held at the tip of the proboscis and this tooth is so fine that even if the poison were to be squirted through it, it is highly improbable that the necessarily fine jet would be seen with the naked eye. The hollow structure of the tooth complemented by the wall of the prepharynx contracted tightly round it actually converts it into a veritable hypodermic needle and the poison cannot get into the victim's body unless this tooth has been driven into its tissues.

BRUCE CUMMINGS (1936) describes an interesting encounter between a cone (*Conus textile*) and a small octopus which can be considered a good example of the use by *Conus* of its poison apparatus in defence against an enemy that is quite numerous in ecological areas where numbers of *Conus* are also to be found. I quote from his paper —

"In the course of seeking material for cinematographic study a small party set out on the exposed reef at Green Island and came across a small octopus whose tentacles extended some 8 or 9 inches from its body. Placing this in an enamel pail of sea-water a further search resulted in the discovery of a live cone shell, *Conus textile*, which was likewise deposited in the same receptacle, where the cephalopod was swimming about freely.

"It was not long however before the latter was aware of the presence of the cone, and some 20 minutes or so later as is usually the case in attacking gastropods, placed one of its tentacles across the entire length of the narrow opening of the shell, with the tip of the tentacle entering further than the remainder. (The mouth of the shell measures  $2\frac{1}{4}$  inches long by about  $5/16$ ths of an inch wide.) About 20 seconds later the octopus quickly withdrew its hold waving its tentacles about with a writhing motion as though violently agitated.

"Inspection of the shell immediately after the withdrawal of the tentacles revealed a thin round spike-like object, evidently the radula, being withdrawn. This spike like radula was about an inch in length, tapering from its proximal extremity to a point distally and was bright red in colour\*. A few minutes later it was noted that the octopus had shed one of its tentacles, it being detached close to its body.

"The octopus was transferred to a glass tank, and although well supplied with abundant fresh sea-water it was found dead on the following morning. On the other hand, the cone shell did not suffer any apparent injury and is still alive and in excellent condition 10 days later.

When the radula is protruded, it is seen directly beneath the siphon, the latter having a red band at its free extremity, a white ring round its centre and a black band proximally. The cone itself measures  $2\frac{1}{4}$  inches in length and an inch and  $3/16$ ths in diameter.

\* Note — This description of the spike-like radula must refer to the proboscis. It cannot apply to the radula which is enclosed in the body cavity and cannot be protruded. The single tooth in use can be protruded, but is nothing like 1 inch in length.

"In view of the reported case of a fatal issue following the bite of a snail of New Caledonia in 1847 from this shell, as well as similar fatalities from other species of *Conus*, this encounter between the two molluscs is interesting. No doubt, such are of frequent occurrence although rarely observed."

### DISSECTION OF *C. CONUS geographus* TO EXPOSE THE POISON APPARATUS.

(Applies equally to *Conus millepunctatus* except as regards the shell.)

Examination of the shell of *C. geographus* containing the animal retracted within it (Plate IV Fig. 1) reveals —

(1) The first whorl of the shell (a) decorated with a distinctive pattern of interlacing brown lines and condensed brown patches forming two or three more or less distinct and slightly oblique transverse broadish bands about equidistantly placed round the shell.

(2) The posterior ends of the remaining inner whorls (b) soldered together and to the first whorl spirally to form the cone-shaped posterior end of the shell, the soldered edges being distinctly wavy in outline.

(3) The animal's foot (c) (seen only in part), the retracted rostrum (d) and the retracted siphon (e)

### ACTUAL DISSECTION

To obtain the animal for dissection it is necessary to remove the first whorl of the shell by cracking it along its attachment to the second whorl with the help of a pair of bone-forceps (Figs. 2, 3). For our purpose it is not necessary to remove the remaining inner whorls of the shell (b) as has been completed in Fig. 4 and Plate III (1 and 2). Indeed, it is advisable to leave these in situ as the spiral whorl of soft tissues (Plate IV Fig. 4 (h) and Plate III, Fig. 1), which consists of various viscera including distal portions of stomach, intestines, liver genital organs, Malpighian tubules, etc., wrapped in a very thin connective tissue capsule is liable to break up into pieces as it is virtually outside the body cavity in a sort of vestibule or recess beneath the floor of the body cavity between the latter and the foot, and hangs rather loosely when the shell is completely removed (Fig. 8, A).

Examination of the animal at this stage on its back and on its foot (Figs. 2, 3, 4 and Plate III) reveals the following parts: foot (c), rostrum (d), siphon (e), eyes (f), root of the penis in the male (g), the mantle (h) containing the respiratory organ or ctenidium (i) and the organ of smell or osphradium (j).

For the purpose of our dissection the animal should now be placed on its foot, pinned down to a board, and the following procedure adopted —

#### Step 1

A transverse linear incision, by means of a scalpel, is made through the posterior end of the mantle (h) just in front of its attachment to the posterior muscular collar (m) of the body cavity shown by the dotted line in Figs. 3 or 4

The mantle, which is unattached along its right edge, is now reflected to the left as shown in Fig 5 carrying with it the siphon (*e*) which is really its modified anterior portion and exposing the full length of the rostrum (*d*) the dorsal wall of the body cavity (*l*) situated between the base of the rostrum (*d*<sup>1</sup>) and the posterior muscular collar (*m*) the full length of the curved penis (*g*) and the two eyes (*f*, *f*). The ctenidium can be seen in the reflected mantle through its inner lining now seen from above (*i*)

### Step 2.

Cutting along the dotted line (in Fig 5) the rostrum is laid open by removing an inverted U shaped segment of its dorsal wall, and exposes to view the proboscis (*n*) with, in many specimens a slender harpoon like radular tooth (*o*) protruding from its tip (Figs. 6 7 8). The proboscis is a tongue-like muscular organ, transversely striated, tapering to a point at its anterior free end and attached posteriorly to a muscular partition or collar (*p*) (Figs 6 7 8 9) which separates the cavity of the rostrum from the body cavity immediately posterior to it. It contains the prepharynx (*q*) which is easily exposed by slitting open the proboscis in the middle line along its dorsal wall from tip to base. The prepharynx is a long slender tube, at this stage apparently attached along its whole length to the floor of the proboscis (Fig 7 and Plate III) but actually occupying a central position in the proboscis (Fig 8 *q*) in which it is suspended by loose bundles of muscle. (Plate, V Figs 1 2 and 3) About 4 mm. from its tip (Fig. 7) the prepharynx shows a small fusiform thickening which corresponds to a strong muscular collar (Plate V Fig 1 and Plate III) evidently meant for gripping the base of the radular tooth in use at the time.

### Step 3

Now laying open the body cavity by an I-shaped incision in its dorsal wall (dotted lines in Fig 6) and reflecting the two halves respectively to right and left (Fig 7 1 *I*) exposes the anterior portion of the alimentary tract—pharynx (*r*) oesophagus (*s*) stomach (*t*)—a solitary salivary gland (*u*) the radula sac (*v*) and the poison apparatus consisting of a long lightly coiled tubular poison gland (*w*) which at the same time acts as the poison duct, and a muscular storage poison sac (*x*) all clearly shown in Figs 7 and 9

The pharynx (*r*) is situated at the anterior end of the body cavity just behind the muscular collar (*p*) which forms the base of the rostrum. It is continuous anteriorly through this muscular collar (*p*) with the prepharynx (*q*) and posteriorly with the oesophagus (*s*) which courses posteriorly to the left on the floor of the body cavity to join the stomach (*t*) which, at the posterior left angle of the body cavity leaves the latter and dips down to join the intestine in the vestibule between the floor of the body cavity and the foot (Fig 8)

The solitary salivary gland (*u*) is situated in the anterior part of the body

cavity to the right of the pharynx. It has two ducts which pass medially ~~outside~~ the radular sac (r) to open into its short arm, one dorsally and the other ventrally (Fig. 9 n)

The *radula sac* (v) is angled or wish-bone shaped, with a short and a longer arm. The shorter arm narrows anteriorly to open into the right side of the pharynx and the longer arm crosses the middle line dorsal to the oesophagus, to the left side of the body cavity where it ends in a cul-de-sac. It contains the radular teeth which are anchored to the angle of the sac, each by a colourless solid cylindrical acellular hyaline ligament slightly longer than the tooth itself (Figs. 9 10 (a) and Plate IV Figs. 1 5 6). The teeth show a dual arrangement in the sac —

Two groups (Fig. 9 and Plate V Figs. 5 6)

1. One group pointing towards the cul-de-sac.

2. The other group pointing towards the opening in the pharynx.

Each group is disposed in two rows, one on each side of the middle line according to the formula x-o-x

Plate IV Fig. 8 is a transverse section of the longer arm of the radula sac showing the two rows in which the group pointing towards the cul-de-sac are disposed.

Each *tooth*\* presents the following characters —

Average length, 1 cm. consists of a sheet of chitin rolled spirally into a cylindrical hollow harpoon (Figs. 10 b c d, and Plate V Figs. 2, 5 and 6) forming a veritable hypodermic needle, flattened at the anterior end into a lancet like point slightly curved near the tip armed on one side with a small barb and on the other side by a blunt ended blade at a level slightly posterior to the barb both pointing backwards (Fig. 10 a, b) on the external surface from the level of the barb for about half the length of the tooth, there is a row of fine hooked denticulations, with points directed backwards, and averaging 130 in number (Fig. 10 a b c e) the base is swollen and slightly thickened in parts (Fig. 10 d) and is attached to a solid cylindrical acellular hyaline ligament (Fig. 9 10 a d) by which it is fixed to the angle of the radula sac. Those in the radula sac are obviously unusable, except perhaps the group pointing towards the opening into the pharynx which may possibly be used to chew up food as it passes down opposite the opening. Many specimens of *Conus* dissected, however show a single tooth in the prepharynx, frequently protruding at the end of the proboscis (Figs. 6 7 8 n and o) and serial sections of a proboscis containing such a tooth, from tip to base, show the tooth followed by the ligament which eventually disappears, showing that when the tooth is transferred to the prepharynx the ligament which is attached to the angle of the radula sac actually snaps. Although not now anchored, the tooth can be

\* (1) BROWN (1895) gives excellent figures of the radular tooth of many species of *Conus*, as also does PERL (1939)

(2) The teeth are apparently not opaque to X-rays (Plate III)

firmly held in a grip by the strong muscular collar placed about 4 mm. proximal to the tip of the prepharynx. In this position two possible functions may be attributed to the single tooth (1) its barbs and hooked denticulations may be used for tearing the tissues of prey on which the animal is feeding (2) its use as a hypodermic needle for injection of the poison into the victim (probably its most important function)

How long this tooth remains in use in the prepharynx it is impossible to guess. The presence of barbs would suggest that it must sometimes remain fixed in the victim's tissues and thus lost to the cone but replaceable by a fresh one from the radula sac. Examination of the sac by blunt dissection to expose the contained teeth shows about a dozen in the long arm of the sac and about three or four in the short arm (Plate V Fig 5). One has no idea of the span of life of a *Conus*, but judging from the size of the shell of larger specimens it might be a matter of years. If a tooth is lost each time the animal stings a prey or an enemy in self-defence, the stock would soon be depleted unless the radula keeps producing new ones at a rapid rate. It is more likely however that each tooth transferred to the prepharynx can be used very many times.

The most intriguing problem is the method by which a fresh tooth is transferred from the radula sac to the prepharynx. At first I believed that the explanation lay in the retractibility of the proboscis which could thus become almost flattened against the collar of the rostrum. If a tooth was pushed into it from the radula sac by some muscular action, with the barbed point just past the surface, while the posterior end was gripped by the strong muscular collar near the tip of the prepharynx, extension of the proboscis would carry it forward until the anchoring ligament snapped. This would leave the tooth in the correct position in the prepharynx.

I presented the problem to Lieut. Colonel A. J. PEILE at the British Museum in 1933 but he could not suggest a better explanation. However a few weeks later I received a very enthusiastic letter from him suggesting a second explanation that of invagination of the proboscis and in November 1943 he wrote to me as follows —

"You may be interested to know that in addition to several cases of the single tooth I have observed in the proboscis of *Conus* I have once found, in *C. terebra*, the proboscis in the position of invagination though it had not actually grasped a tooth.

I have drawn Fig 9 to represent this action of the proboscis diagrammatically. Serial sections of the proboscis (Plate V Fig 1 2 and 3) show a very loose arrangement of muscle bundles between the external wall of the proboscis and that of the contained prepharynx suggesting a great power not only of retractibility but also of expansion, so that invagination is feasible and, in view of Colonel PEILE's observation mentioned above, is probably a reasonable mechanism.

In the first explanation some muscular action from the radula sac or

pharynx would be necessary to drive the tooth forward past the retracted proboscis before it could be drawn forward. In the second theory this would be unnecessary as the tip of the proboscis could be invaginated right back into the pharynx to the opening of the radula sac.

The most likely explanation, however is probably a combination of retraction of the greater part of the proboscis with invagination of its anterior tapering segment, thus reducing the length of invagination required to reach a tooth presenting in the pharyngeo-radular opening.

The *poison sac* (*x*) is a curved, pear shaped, firm and solid looking but hollow muscular organ measuring 20 mm. by 7 mm. (Figs. 7 8, 9 16 17 Plate III and Plate V Fig 7) occupying the posterior half of the body cavity in a transverse position, with the narrowed anterior end to the right where it joins the coiled tubule. Its lumen in transverse section is nearly circular (Fig. 8 and Plate V Fig 7) but elongated and curved in longitudinal section (Fig. 9). The wall of the sac consists of four coats, which from the outside inwards are —

- (1) A thick outer muscular coat with fibres disposed in various directions, oblique, longitudinal, but mostly circular. It is about four to six times as thick as the other three coats taken together.

- (2) A thin connective tissue coat sending fine shreds into (1).

- (3) A longitudinal muscular coat thinner than (2).

- (4) A thin connective tissue basement membrane lined by a single layer of non-ciliated, non-granular cubical epithelial cells.

The cubical epithelial lining does not suggest a secreting epithelium, and the sac would appear to be merely a storage sac and propulsive organ for poison probably secreted by the coiled tube now to be described.

The *tubular poison gland* (*w*) is a highly coiled tube approximately 75 mm. long, occupying the space of the body cavity behind the radula sac and between it and the poison sac, dorsal to the oesophagus. Its coils are bound down by very loose connective tissue. Posteriorly it arises from the narrower anterior end of the pear shaped poison sac, and anteriorly it passes forward, ventral to the long arm of the radula sac to open into the left side of the pharynx. A transverse section (Plate V Fig 4) shows the following structure —

Circular shape.

External wall of longitudinal muscle.

Intermediate layer of circular muscle.

Thin connective tissue basement membrane.

Internal epithelial lining made up of several layers of elongated columnar cells with basal nuclei and cytoplasm rich in granules. It varies in thickness at different parts of the circumference, rendering what is left of the lumen irregularly star-shaped.

The *poison sac* (*x*) is known among zoologists as the "poison gland of Læblich" and the coiled tube as the poison duct. In their description of *C. striatus* CLENCH and YOSHIO KONDO (1943) describe them under the name

of "Simroth's Giftdrüse" I venture to suggest that the pear-shaped muscular sac with its non-granular cubical epithelial lining is not a poison gland at all but merely a storage and propulsive organ for the poison, and that the latter is actually secreted by the coiled duct which is lined with high columnar epithelium of glandular secreting type as shown by the large numbers of granules present in the cytoplasm of the cells again, if it was merely a duct its great length and highly coiled state would be unnecessary. This, however does not prevent it from acting also as a duct through which the poison is propelled into the pharynx by the contraction of both the muscular sac and the muscular wall of the coiled tube itself.

With the base of the hollow radular tooth firmly gripped by the muscular collar of the prepharynx one can easily imagine how the poisonous secretion coming along the pharynx and prepharynx under pressure is forced through the hollow tooth into the prey to immobilize it before the meal is started, or into an enemy for the purpose of self-defence.

#### ASSAULT OF *Conus* ON ITS VICTIM.

There appears to be a contradiction in terms in the description of the assault by *Conus* on its victims. Most writers describe it as a bite, and some describe it as a sting. The term bite cannot be correct for a radular tooth is not morphologically or even functionally comparable with a tooth, which is strictly a vertebrate structure. The radula is not part of the jaw which, when present in mollusca, is anterior to the radula. The word "sting" is obviously more suitable and descriptive of the action.

Concerning the assault by *Conus* after removal from the sea, it would be interesting if future workers in this field devoted some attention to the type of stimuli responsible for the reflex. ALPERS (1932) tried in vain to make *C. mediterraneus* sting his fingers.

#### SUMMARY

Marine gastropods of the genus *Conus* have been recorded for nearly a hundred years as having caused serious and even fatal accidents by stinging human beings, but have so far found no place among the venomous animals dealt with in textbooks of tropical medicine.

Prior to 1932 all such accidents of which five proved fatal, were confined to the Western Pacific. A fresh case of stinging by *Conus geographus* Linn., fortunately not fatal, is now reported from the Seychelles Islands and is the first case recorded from the Indian Ocean.

The method by which the animal introduces its poison into the body of its victims has so far not been directly observed but a definite apparatus which probably secretes the poison, and is adapted for its ejection, is revealed by anatomical dissection and histological study and there is good circumstantial



evidence to show that the poison is injected through a detached tooth from the radula used as a hypodermic needle

Histological study further reveals that the so-called "Gland of Leiblin" is not a gland at all and cannot be the source of the poison (except indirectly by acting as a reservoir for it and as a muscular propelling organ for its ejection). The source of the poison is almost certainly the coiled tube previously regarded as the duct of the "Gland of Leiblin" being lined by tall, secreting epithelium, it is really equivalent to a tubular gland which can, however at the same time, act as the poison duct. This is a new conception.

Attempts to verify the effect of the poison on mice with extracts (in 30 per cent. alcohol) of the poison from the poison apparatus of *Conus geographus* Linn., and of *Conus leopardus* Röding (= *millepunctatus* Lamarck) after prolonged storage in the tropics, failed either through inactivity of the extract or through refractoriness of the mice used. It is accordingly suggested that a fresh series of experiments might be undertaken by some worker in the tropics, where the poison could be obtained and used in the fresh condition.

Since the effects of *Conus* poison appear to be opposite to those of the poison secreted by the gland at the base of the venomous spine of the dorsal fin of fishes of the genus *Sympterna*, investigations on the nature and effects of the former might profitably include attempts to confirm this notion. Observations might also be made on the nature of the stimuli able to produce the stinging reflex in *Conus* particularly when handled.

Original work on the anatomy macroscopic and microscopic, of the poison apparatus of *Conus geographus* and *Conus leopardus* not previously attempted, is described and illustrated.

As full extracts of reports on actual cases of cone stings and their sources of publication are given in the form of an Appendix, these have been omitted in the usual list of references at the end of the paper

It is a pleasure to acknowledge the assistance, in the first place of Mr JOHN TYSON, of the Cable and Wireless Co., Ltd., Miss ANNE DE LAFONTAINE and Mr FRIEDRICH DE LAFONTAINE, in making it possible for me to complete the relevant part of the anatomical work and to attempt (though unsuccessfully) to elucidate the character of the poison of *Conus* of Mr J. W. BAGGALLY Curator of the Sheffield City Museum, in lending me some specimens of cone shells of which I was short for the illustrations, my specimens having been left in a large collection of shells I presented to the Carnegie Library in Victoria, Mahé Seychelles, as a nucleus for a Natural History Museum in the colony, of Dr A. TINDALL HOWARD Mr J. RILEY B. TOMLIN Professor ERNEST FINCH and Dr E. T. B. FRANCIS for much help in obtaining the literature of my colleague, Dr O. EXCLAMER, for the translation from the German, of the observation of F. ALPHEUS on *Conus senhaverensis* of Mr N. W. BROWN my technician at the Royal Infirmary in preparing the numerous serial histological sections and Mr G. S. EVANS, my head technician, in preparing the photographs and illustrations, except those on Plate I the first two of which were supplied by Mr FRANÇOIS VEL, Seychelles, and the third taken specially for me by Mr JOHN TYSON

Plate V was originally intended to appear in colour and had been beautifully executed by my friend, Mr CHARLES DYSON, Sheffield, but owing to high cost of colour reproduction at the present time it has had to appear in black and white.

My very particular thanks are due to Lieut.-Colonel A. J. PELLE for many valuable suggestions by correspondence, and after reading my paper introducing it to Mr. R. WINCKWORTH, whom I cannot adequately thank for the trouble he spontaneously took to criticize my paper so minutely that I feel he should be part author. To him I am also indebted for all the complete up-to-date list of cones from the Seychelles Archipelago with literature.

I must, finally acknowledge the help of my secretary Miss KATHLEEN GREENWOOD who very willingly spent many weary hours typing and retyping this article.

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## APPENDIX I

SPECIES OF *Conus* RECORDED FROM THE SEYCHELLES.

Authentic records from the Seychelles Islands are numbered. Other records require verification. Records from the Amirante Islands are in brackets and marked a from Coctry in brackets and marked x.

W = collected by H. C. WINCKWORTH. H = collected by HERMITTE. d = DUFO  
 l = LIÉNARD m = MÖBIUS D = DAUTZENBERG S = MELVILL (*Sealark*)

	Species	Records.	Remarks	Group
1	<i>C. arenatus</i> Bruguière	W.H.d.L.(Sa)		<i>Puncticulis</i>
	<i>C. pulicarius</i> Brug	d.		"
2	<i>C. ceylanicus</i> Gmelin	W d. l.	= <i>obesus</i> Brug	"
3	<i>C. ebraeus</i> L.	W.H.d.l.m.	Typical and form <i>chaldaeus</i> (Röding)	<i>Vitroconus</i>
4	<i>C. coronatus</i> Gm.	W.d.l.(Sx)V	= <i>willmeri</i> Brug	" ?
5	<i>C. pusillus</i> Dillwyn	W d.l. Nevill	Not <i>pusillus</i> Lam. It has no correct name.	<i>Vitroconus</i> ?
6	<i>C. ceylanensis</i> Brug	W	Var with reduced markings	" ?
	<i>C. musicus</i> Brug	d.D	Perhaps intended for 6.	" ?
7	<i>C. betulinus</i> L.	H.d.m.D		<i>Cleobula</i>
8	<i>C. figuratus</i> L.	H.(da)		"
9	<i>C. quercinus</i> Solander	H.(da)D.S		" ?
10	<i>C. brevis</i> Lamarck	W.d.m.D.(Sx)		<i>Lithoconus</i> ?
11	<i>C. flavus</i> Brug	W.d.m.		" ?

	Species.	Records.	Remarks.	Group.
12	<i>C. rufus</i> Brug.	W.d.		<i>Rhissocoma</i>
13	<i>C. capensis</i> L.	H.(da)l.		
	<i>C. senegalensis</i> Brug.	Klener	Only Seychelles record = <i>senegalensis</i> Lam.	
14	<i>C. erinacea</i> Born.	H.(da)D		
	<i>C. seychellensis</i> Nevill	Nevill	Insufficiently described near <i>erinacea</i>	
15	<i>C. vexillum</i> Gm.	W.J.S.		
16	<i>C. miles</i> L.	W.H.d.l.		<i>Lithacoma</i> ?
17	<i>C. turgo</i> L.	W.H.d.		?
	<i>C. mactans</i> Croas	Croas	From Mus. Cuming : Cuming records are unreliable, = <i>caerula</i> Swanson	
	<i>C. circumscissus</i> Iredale	(l.) (Sa)		
	<i>C. turbinatus</i> Brug	(lk)		
	<i>C. teretis</i> Born	l.		<i>Leptacoma</i>
	<i>C. glauca</i> Brug	W	One dead shell	
18	<i>C. mactans</i> L.	W.d.l.(Sa)		<i>Hermes</i>
19	<i>C. leoparatus</i> (Röding)	W.H.d.D	= <i>molleporatus</i> Lam.	<i>Lithacoma</i>
	<i>C. littoralis</i> L.	(da)D		
	<i>C. eboreus</i> Brug	(da)		
20	<i>C. tessellatus</i> Born.	W.H.d.l.m.D.(Sa)		<i>Leptacoma</i>
21	<i>C. generalis</i> L.	W.H.d.	All records are of form <i>maldianus</i> Brug.	
	<i>C. varius</i> L.	(da)		
	<i>C. brachy</i> Sowerby	Sowerby	Only record	
	<i>C. archilobatus</i> Sol.	(Sa)	Probably error of identification	
	<i>C. eximius</i> Reeve	(Sa)	Perhaps young <i>generalis</i>	
22	<i>C. marmoratus</i> L.	d.m.		<i>Coma</i>
23	<i>C. imperialis</i> L.	W.H.d.		<i>Rhombus</i>
24	<i>C. distans</i> Brug	W	On several occasions, all living	
25	<i>C. talpa</i> L.	W.H.d.D		<i>Gastrolites</i>
26	<i>C. geographicus</i> L.	W.H.d.l.m.D.		
27	<i>C. bullatus</i> L.	H.l.		
	<i>C. macula</i> Brug	(da)		<i>Cylindrus</i>
28	<i>C. suber</i> L.	W.H.d.D		
	<i>C. opicopus</i> Brug	(da)D	Perhaps a confusion with young <i>suber</i>	
	<i>C. textile</i> L.	d.l.	True <i>textile</i> does not seem to occur in the Seychelles but the related species are often confused with it.	
29	<i>C. cuneatus</i> Brug.	W.d.D.		<i>Chelyconus</i>
30	<i>C. catus</i> Brug.	W.d.(Sa.)		

Species.	Records.	Remarks.	Group.
<i>C. frigidus</i> Reeve	D	Possibly <i>catus</i> var	<i>Chelyconus</i>
<i>C. pertusus</i> Brug	LD		"
<i>C. strictus</i> L.	I.		<i>Dendroconus</i>
31 <i>C. gubernator</i> Brug	W.H.d.I.(Sa)		"

In addition to the above, the following species which are not even Indo-Pacific have been recorded from the Seychelles or Amirantes —

<i>C. papilionaceus</i> Lam.	(d)	This is West African.
<i>C. hyens</i> Lam.	d.	West African.
<i>C. prometheus</i> Brug	Reeve	West African.
<i>C. mundanus</i> Brug.	I.	West Indian.
<i>C. nebulosus</i> Lam.	(da)	West Indian.

#### LITERATURE ON SEYCHELLES MOLLUSCS

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- D. DAUTZENBERG P. (1893) Contrib. à la faune malacologique des îles Séchelles. *Bull. Soc. Zool. Fra.*, 18. A rather short list of shells received from several collectors. I do not doubt DAUTZENBERG's identifications but I strongly suspect the list includes material not only from the Seychelles but also the Amirantes, Aldabra, etc.
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- DUPORE R. (1935) La faune des Seychelles 5 Crust. et moll. *Trans. R. Soc. Maur.* C.3 [Not seen.]

#### APPENDIX II

##### SUMMARY OF LITERATURE ON CONE STINOK. 1848-1943

ADAMS, A. (1848) *Narrative of the voyage of H.M.S. Samarang* 2 pp 356-357. Also in ADAMS and REEVE, 1848. *The Zoology of the voyage of H.M.S. Samarang*. Mollusca, p 19.

"The animal of *Conus aulicus* has the proboscis beautifully varied with red and white, and there is a square and very minute operculum on the dorsal surface of the hinder part of the foot. Its bite produces a venomous wound accompanied by acute pain and the making a small deep triangular mark, which is succeeded by a watery vesicle. At the

little island of Mayo, one of the Moluccas, near Ternate. Sir EDWARD BELCHER (commander of the *Somerset*) was bitten by one of these cones, which suddenly exerted its proboscis as he took it out of the water with his hand, and he compares the sensation he experienced to that produced by the burning of phosphorus under the skin. The instrument which inflicted the wound, in this instance I conceive must have been the tongue, which in these molluscs is long and armed with two ranges of sharp-pointed teeth."

GRAY J. E. (1853) On the Head of the Genus *Coccul* Linn. *Ann Mag nat Hist.* (2) 12, p. 178.

After quoting ADAMS' account given above he adds "Mr ADAMS informs me that it adheres to the hand by its mouth like a leech, as described by ADAMSON."

MACGILLIVRAY J. (1860) Zoological Notes from Aneityum, New Hebrides. *The Zoologist* 18 pp. 7136-7138. On a poisonous property attributed to *Coccul textile*.

"On my first visit to Aneityum I was told of a shell-fish which, on being accidentally handled, is said to eject a poison, causing if it comes in contact with the hand, an immediate and peculiar sensation, then numbness of the hand and arm, followed by intense pain, usually severe illness, and not infrequently death. The native name is Intrag, and the mollusc in question is the well-known *Coccul textile*. Having frequently handled this shell-fish while collecting on coral reefs in the Pacific, Torres Strait, and the north-east coast of Australia, without having sustained any injury from it, I was naturally somewhat incredulous in the matter yet as the general belief—which is never wholly destitute of foundation, was against me I yielded to it so far as afterwards to handle with caution any live specimens I saw. I was told that the small intrags and those of certain localities (one of which is near my present residence) are reputed more dangerous than others. The intrag is not usually considered dangerous unless the animal be touched, which of course no one here will do, except unwillingly but some of the natives say that it can blow the poisonous influence upon the hand of an intruder from the distance of several inches.

"On 9th June of the present year about 10 p.m., I had brought to me a young man, my neighbour Nivenham, who was said to have recently been poisoned by the intrag, and appeared to suffer intense pain. From what I could learn it seems that he and a companion had been looking for shell fish by moonlight about 2 hours previously. N. had picked up in the shallow water something which he did not see distinctly. Immediately on touching it, and while his hand was in the water he felt a sensation as if some very cold water had been blown on the palm of his right hand, and dropped the object, which he saw was an intrag. Not long afterwards he went home and soon began to complain of numbness in the whole of his right arm and hand. This was immediately ascribed to his having touched the intrag and his companion went back to the spot for it, carefully picked it up, the shell with the animal retracted, and eventually it was given to me. A bandage was tied tightly round the sufferer's arm at a little below the shoulder, and when I saw him the arm was cold and much swollen, and the pulse about 50 and very feeble. I administered an enormous dose of the solution of muriate of morphia, as he was suffering excruciating pain. A medical man in New Zealand having suggested the experiment of burying the hand and arm affected in fresh earth, this was done but the patient could not endure it for long, for he literally writhed with pain while lying on his face on a mat, with his arm in the ground. Meanwhile a man experienced in such matters had been sent for. On arrival he prepared a knife of two strips of bamboo, and made two deep incisions in the upper part of the arm, one in front, another behind, below the ligature which had been slackened. About half a pint of blood was obtained. Next morning at 8 a.m., I found that the morphia had produced sound sleep during the night, and that the bandage had been removed according to my suggestion. The right arm was swollen and felt rather cold, but the pulsation was equally strong at each wrist, sixty-three beats to the minute. Means were taken to assist in restoring the normal temperature to the arm, and wine was ordered, to be discontinued on indications of reactions showing themselves. All pain, except from the incisions, had disappeared and in the course of about a week the patient recovered his usual health. With regard to this case it is right

to mention that although satisfied by the circumstantial evidence that contact with an intrag had produced extraordinary effects, yet I could not separate them satisfactorily from those attributable to the ligature. No pain was felt before the bandage was applied.

"A case which terminated fatally may now be mentioned. On 28th May 1859 I went along with the Rev J GEDDIS to see a sick woman, who 14 days before was believed to have been poisoned by her hands having accidentally come in contact with an intrag while collecting shell fish on the reef. The whole right hand and arm to within a few inches of the axilla were in a state of gangrene with the bone exposed in several places. No hæmorrhage however had taken place. I could see that numerous small but deep incisions had been made in the arm. There was not, I may mention, as with a light skinned person, the same facility for ascertaining the existence of a line of demarcation between the living and the dead portions of the body. Apparently there was some material to render amputation at the shoulder-joint possible, but, unfortunately on the back of the shoulder also on the sides of the chest, there were indications of incipient gangrene in the peeling off of the cuticle and the formation of vesicles, rendering the operation inadvisable because holding out no hope of saving life. In this case I learned that a tight bandage had been kept on for several days, probably of itself sufficient to induce mortification even in a healthy limb.

These two cases are the only ones of which I can say anything from personal observation, and I shall make no further comment than merely to observe that as I cannot find any special apparatus in the animal of *Conus textile* or see any anatomical difference between it and *C. arenatus* (which is known to be innocuous) after examining both I feel great reluctance in subscribing even to the universal popular belief on this island of the power of the intrag to cause injury to man in the manner ascribed to it. A jet from the siphon of the animal might partially account for the first sensation experienced. No puncture or abrasion of the cuticle is ever spoken of but in some cases, I have been told, the skin has been discoloured the word used being *emilmat*, which means either blue or green."

BENNETT GEORGE. (1860) *Gatherings of a Naturalist in Australia* London. Footnote, p 382.

"The common *Conus textile* of Linnaeus is found at Anestum, and other islands of the New Hebrides' group the animal is poisonous on biting its captor it injects a poisonous and acrid fluid into the wound, occasioning the part to swell and often endangering the life of the injured person."

CAOSSE, H. & MARIE, E. (1874) *J Conchyliol.* 22 p 353 (translated by JOSEPH BEQUAERT for WILLIAM J CLENCH and YOSHIO KONDO 1943 *Poison cone shell. Amer J trop Med* 23, 1 p 114).

(Under *Conus tulipa*)

"According to Dr MARIE, the sting of the animal *C. tulipa* is as venomous as that of *C. textile*. It is by error however that the bite has been blamed on the operculum it is made by the armature of the tongue.

(Under *Conus textile* Linn.)

"This species reaches in New Caledonia, a very large size. The fact mentioned before by several English naturalists, that the bite of *C. textile* is venomous, was confirmed in New Caledonia. According to an eye witness, a native of Pouébo after being stung on the hand, suffered a considerable swelling of this hand and the corresponding arm, with very sharp pain. The swelling persisted for some time. However the mistake was made in that country of blaming the operculum of the *Conus* for what was caused by the teeth of the tongue."

MOUTROUZIER, R. P. (1877) *J Conchyliol* 25 p 99 (translated by BEQUAERT for CLENCH & YOSHIO KONDO, 1943 *Poison cone shell, Amer J trop Med.*, 23 1 p 114)

"We received from Father MOUTROUZIER, our correspondent in Noumea, a communication which seems to confirm the truth of the venomous properties of the lingual teeth of species of *Conus*, as claimed by some naturalists. Father MOUTROUZIER writes us that at Maré, one of the Loyalty Islands, *Conus marcorum* which is abundant there,

cannot be handled carelessly without the risk of causing accidents through the sting of its tongue. In the New Hebrides accidents due to the sting of *C. textile* are said to be rather frequent."

GARRITT A. (1878) *Annotated Catalogue of the Species of Conus Collected in the South Sea Islands*. *Quart J Conchol.*, 1 p. 365. Under *Conus talipa* Linn., he has the following notes:

Somewhat plentiful under clumps of coral on reefs. When collecting at the Paumotu, I found three examples of this species, and held them in my hand while searching for other shells, when one suddenly threw out its long slender proboscis and punctured one of my fingers, causing sharp pain not unlike the sting of a wasp."

He records *C. talipa* from the Viti, Tonga, Samoa, Kingman, Caroline, Cook, Society, Paumotu, Marquesas and Sandwich Islands.

COX, J. C. (1884) *Poisonous Effects of the Bite Inflicted by Conus geographus* Linn. *Proc. Linn. Soc. New South Wales* 9 pp. 944-946.

In the above article is published the following letter received by Dr Cox from Mr B. H. HINDS, R.N. Surgeon on H.M.S. *Diamond*.

H.M.S. *Diamond*,

"At sea, Lat. 10 14'S., Long. 155 34'E.

"The following facts which I have learned partly by hearsay and partly by personal observation, concerning the shell, known as *Conus geographus* of Linnaeus, may be of interest.

"What first drew my observation to this curious power of *C. geographus* was, a native of Nodup New Britain, an interpreter on board H.M.S. *Diamond* seeing me with a specimen of *C. geographus* in my hand remarked, 'suppose he bite he kill man.' Thinking this to be an exaggeration on the part of the native, but at the same time thinking that he must have some reason for so saying, I enquired of him more particularly as to how the shell would harm anyone as at the time I fancied that he meant if the edge of the shell cut a person by accident it would cause blood poisoning; however he described how that the fish would bite and that the bite was poisonous, and that it always killed people if they did not cut themselves to let the blood run, all round the place bitten. He also promised to procure me a live specimen and show me how it bit.

"This promise he carried out as nearly as he could for he brought me the shell, but said when he went to take it up the animal had retired, or rather commenced to retire, into its shell when he cut off the head, which he brought me separated from the shell. The shell he brought was about 5 inches in length.

"Some time afterwards, being in conversation with a Mr R. PARKINSON, a New Britain cotton planter I enquired if he knew anything of this man's statement about this *Conus*. He told me that he believed it to be perfectly true, and that he had written about it to someone in Sydney. I should have taken no more notice of the statement but for the fact that I saw myself a native, on the island of Matupi, Blanche Bay New Britain, who had been bitten by one, and who had at once cut small incisions with a sharp stone all over his arm and shoulder from which the blood had flowed freely and he explained to me that if he had not taken these precautions he would have died. He explained to me also the shell and how he had been bitten (there was a mark about the size of a three-penny piece) between his finger and thumb, but upon close examination there were two small incisions in the centre but from which evidently no blood had come.

"I may mention that to stop the bleeding of the numerous cuts in his arm and shoulder hot wood ashes had been put on them, and the arm seemed to be stiff and useless for the time. But whether this was the effect of the bite or the cure I really am unable to state.

Many natives whom I questioned (showing them the shell at the same time) said that the bite was deadly.

"Hoping that these few observations may be of use either as information or confirmation to Conchologists generally

"BING HUGH HINDS, R.N.

Dr Cox further states in the article that an instance had been recorded by Mr ARTHUR ADAMS of a poisoned wound produced by the bite of *Conus anfract* Linn., that the

Rev W WYATT GILL\* had recorded the fatal effects of the bite of *Conus textile* Linn. that Mr BRAZIER had informed him that he had known severe effects caused by the bite of *Conus talpa* Linn. and that the above was the first instance he (Dr Cox) had heard of the poisonous effects of *Conus geographus*

HEDLEY C. (1892) *British New Guinea* by J P THOMPSON London Appendix pp. 283-284

"The natives are quite aware of a poisonous bite inflicted by several of the cones. While collecting on a coral reef I once rolled over a boulder and exposed to view a living *Conus textile*. Before I could pick it up one of my coloured companions hastily snatched it away and, pointing to its business-end, explained with vivid gesticulations its hurtful qualities. He would on no account allow me to handle the shell but insisted on putting it himself into my bottle of spirits."

COXON Mrs. C. (1894) Notes on Poisonous Cones. *Proc Royal Soc Queensland* 10 pp. 38-39

Mrs. COXON after quoting from the above earlier reports, adds the following note "This account is borne out by a specimen received from Tanna (New Hebrides) My late husband sent £2 to a missionary at Tanna for shells. In one of those (a *Conus*) which he received from the missionary is a memorandum—written I suppose, by the sender—stating that the animal sometimes bites its captor and injects a fluid poison into the wound which causes death in a few hours through contraction of the throat.

COCKER, A. H. (1895) *Cambridge Natural History* 3 Mollusca, pp. 65-66

In the chapter on *Conus*, COCKER states that "The poisonous nature of the bite of certain species of *Conus* is well authenticated" and quotes a few passages from HENDER MACGILLIVRAY ADAMS and HEDLEY which are quoted in full above.

HALLIDAY Dr A. HERBERT (1901) Government Medical Officer's Report, Levuka, Fiji, for the month of June.

An extract of her report, given in detail below was forwarded by Dr B G CORNEY from Fiji, 10th September 1901 to the Australian Museum, Sydney Accompanying was a shell said to be similar to the one that had inflicted the severe bite described in the report. The shell was identified as that of *Conus geographus* Linn.

"I had under observation a case of a European lady here who was the subject of a severe form of poisoning by a shell fish of the species of which a shell is now sent for identification.

"The lady was fishing not far from the shore in the evening with her family and native servant in the boat. The shell fish having been obtained, the boy cracked it to extract the meat, which was large in quantity for the size of the shell and having cracked the shell handed it to his mistress with the meat hanging from its internal attachment. To free the flesh, she inserted her little finger towards the upper end and she declares, she felt it shoot out a sharp-pointed thing which penetrated her finger and caused such a peculiar sensation that she at once called out that she was bitten and poisoned.

"The poisonous matter is said to be yellow pulpy matter at the thicker end of the shell it might, of course, be merely reproductive or digestive tissue, or again there might well be a modification of some secretory gland to form a protective poison gland, and in the latter case nature would surely provide along with poison some mechanical means to promote injection into the enemy

"The point of puncture in this case was minute and only to be seen with great care indeed, that it was a puncture was much less readily seen than the local effect of the poison which caused a bluish discoloration of the surrounding tissue. It was situated at the point of the patient's little finger near the side of the nail. Through so small a puncture, and in so short a time as was allowed to its insertion (she did not, unfortunately suck the

\* See H. FLECKER, 1938 quotation by CLELAND (1912) from Rev W WYATT GILL's *Life in the Southern Isles*



wound), but a most minute quantity of the poison could have entered the circulation, yet the effects were most grave. Locally a numbness was first experienced. This extended rapidly up the arm, which became paralysed and the paralysis spread thence rapidly throughout the body. It was peculiar that not only was general muscular control abolished, even so far that the head had to be supported over the trunk in order that unimpeded breathing might be allowed to continue, but there was a loss also in a lesser degree (as I think) of sensation, with numbness and pins and needles beginning in the arm and becoming generalized through the body and to a more marked degree. There was a disappearance of muscular sensation and a complete absence of knee jerks. The patient constantly asked where her limbs were. Utterance was thick and indistinct. The respiratory and cardiac muscular apparatus did not at any time participate to a dangerous degree in the paralysis. The stomach, however, may have been affected, for I could not induce vomiting. When at its worst, some 3 or 4 hours after the poisoning began, the condition distinctly affected the throat, and a good deal of distress was caused by the difficulty in removing accumulated fluid. The poison seemed to me to clearly belong to the class of which Curare is the type. Of this I felt assured as soon as I had examined the patient and observed the freedom of the respiratory and circulatory centres from its actions compared with the absolute abrogation of voluntary muscular power so that, the patient weighing 16-odd stones, I felt a good deal of anxiety as to whether the arms would not dislocate at the shoulder when the body was lifted in the chair by the hands under the armpits. Indeed, it was exceedingly difficult to move the patient, all the parts being so abnormally yielding. The treatment I adopted was merely directed to the maintaining of life till the poison should have been destroyed. The heart and lungs were quite equal to their work if other circumstances could be kept favourable. This was done by placing the patient in a semi-recumbent position in a canvas chair and by keeping the head in such a position that breathing and swallowing were facilitated. I should have liked to relieve the circulation by inducing vomiting but failed to do so. Had I any strychnine with me I should have injected it hypodermically but I did not feel justified in leaving the patient to get it. The worst was past in about 6 hours. The wound was made about 9.30 p.m. Paralysis lasted on with steadily diminishing intensity till late next day but the numbness lasted considerably longer in the injured finger and for a month after the patient experienced a shock in the little finger on hard inspection—as in playing the piano. This was the last symptom to clear up unless the sore eyes, which began and lasted later, are to be attributed to this poison as their cause. Though natives declare that recovery from fish poisoning is often complicated by sore eyes, yet I am not aware that the tradition would apply to this kind. I have heard since of other cases of this kind of fish poisoning, and among others, of a Hadaru woman who died before she could get from the shore."

CORNEY B. G. (1902) *Nature* 65 p. 193.

"I notice that doubt is cast on the opinion held by some authorities that the bite of certain species of *Conus* is poisonous and as a case has now occurred here in a European subject whose intelligence places her account of it beyond question, I think it may be useful to represent the corroborative evidence thus obtained.

I should mention, first, that a shell exactly similar to the one in question forwarded to the Australian Museum, Sydney and that I am indebted to Mr. ERMINGHAM, the Curator for information on the point and for the identification of the specimen as the shell of *Conus geographus*.

Then follows a précis of Dr. A. HERBERT HALLÉN's case (1901), quoted as Mrs. R.

CLIFLAND C. BURTON (1912). Injuries and diseases of man in Australia attributable to animals (except insects) *The Aust. med. Gaz.*, 32, pp. 269-274. 295-299. (Phylin Mollusca, pp. 272-274.)

"Bites of shell-fish of the genus *Conus*. Through the kindness of Mr. CHARLES HEDLEY F.L.S. of the Australian Museum, Sydney who has kindly placed the following references to bites from shells of the genus *Conus* at my disposal, I am able to submit a number of valuable accounts of the severe effects produced in man by careless or inexperienced handling of these animals. Save that one of the implicated species is found

along the Great Barrier Reef the subject is hardly to be considered as strictly Australian but, in view of the interest attached to the observations it seemed well to take this opportunity of bringing the references together. I am also much indebted to the courtesy of Mr R. ETHERIDGE, Curator of the Australian Museum, for permission to use the very valuable information supplied by Dr CORNEY the original of which (Dr HALLEN's report quoted in full above) is filed amongst the Museum Records."

The paper gives in full all the various extracts given above and for the first time the full account of Dr HALLEN's case which I have reproduced from it.

This latter case was extracted from CLELAND's paper and reproduced in full in the *Nautilus* 25 pt. 10 pp 117-120 1914 under the title "Poisoning by the Bite of *Conus geographus*" and more recently reprinted in full in the paper by W. J. CLENCH and YOSHIO KONDO (1943) which is referred to below.

SCUTTANT F. (1930) On the poisoning by the bite of *Conus geographus* Linn. *Venus* 2 pp 151-152.

This paper is written entirely in Japanese. Although it is available in the British Museum, I was unable to obtain a translation. According to CLENCH and YOSHIO KONDO (1943) a specific case is given, and then follows a review of this subject, mainly of Dr HALLEN's case given above in detail for CLELAND (1912).

IREDALE, TOM (1935) Fatal sting by a cone. *Nautilus* 49 p 41 and *J. Conchylol.*, 70 p 264 and *J. Conchol.* 20 p 166. Also in *Venus* 5 p 294 (in Japanese).

These four articles give three versions of the same case. The following is a combined summary.

"Although there have been many reports on attacks by *Conus* from the outlying islands of the Pacific Ocean, there has never been previously a case from Australia.

"On 25th June, 1935 a young man in his twenties, CHARLES GARRETT was fatally stung by a Cone, almost definitely *C. textile* L. at Hayman Island, one of the Whitsunday group of islands, Queensland. Apparently he was handling it when a spike came out and pierced his hand. He did not complain of pain until later when he said his sight was failing and he had a burning sensation round the mouth. He grew steadily worse and died whilst being taken to hospital. This is the first case in Australia and there do not seem to have been many others elsewhere."

After quoting briefly references from CLELAND's paper (1912) and listing the five species of *Conus* responsible for attacks on man, he adds "It is possible that all Cones are more or less poisonous but generally the animal is sluggish and does not move much. *Conus textile* is not uncommon on the Great Barrier Reef and many have been collected alive without injury but more care will be taken in the future."

\* Note.—The shell that stung CHARLES GARRETT fatally has since been identified by Mr H. A. LONGMAN of the Queensland Museum, as *Conus geographus* (H. FLECKER, 1936 see below) the actual specimen being now in the Queensland Museum. Two photographs appear in FLECKER's paper which also gives a much fuller account of the case, including results of a postmortem examination.

ALLAN JOYCE. (1935) Poisonous Shell fish. *Med J Aust.*, 1935 pp. 554-555 6 text figures. (Also in *Aust Mus Mag.*, 5 pp 393-394)

"This paper apparently prompted by TOM IREDALE's report of the first case of *Conus* poisoning in Australia (CHARLES GARRETT at Hayman Island, off the coast of Queensland, 25th June, 1935) is a review or summary in general terms of poisoning by *Conus*, based on the above paper and that by Dr CLELAND (1912). A short description of the ecology of Cone shells is followed by a list of the five species of *Conus* so far known to have caused accidents to human beings in the South Pacific Islands—New Guinea, New Hebrides, New Caledonia, Tonga, Samoa, Fiji, New Britain, the Carolines, and the Society Islands and Loyalty Islands.

The manner of attack of *Conus* on its victims is described as extraordinary. "Cones are predatory animals which prey on other shell-fish and suck juices from their shells. They are able to do this by means of a long tubular fleshy structure known as proboscis which can be retracted at will, but when extended reaches beyond the anterior end of the shell.

Opening into this tube is a radula-sac containing two rows of numerous teeth and a bundle at the end. When grabbing food or when opposed by any outside agency such as the hand of a human being, the Cone immediately shoots out this proboscis and the object is pricked by the sharp points of the teeth which are hollow and have a swelling at their base. The poison reaches them from a special poison gland."

The symptoms of poisoning as described are those displayed by Dr HALLÉN's case, (1901) and of that of TOM IREDALE's (1905) (given in more detail by FLECKER, 1936).

The article ends up with the statement that to the author's knowledge there has been no literature published on the proper treatment for this poisoning, and the suggestion that there is ample opportunity for a medical man, interested in this branch of his science, to investigate the poison of Cone shell-fish and its treatment.

FLECKER, H. (1936). Cone shell mollusc poisoning with report of a fatal case. *Med. J. Aust.*, 1936 pp. 464-468, 2 text figures (photographs of *C. geographus* which caused the fatality).

In this paper the author quotes most of the cases given by CLELAND (1912), and adds new material as follows —

"(a) CLELAND Sixth report of the Microbiological Laboratory (New South Wales Government Bureau of Microbiology) 1915

Professor CLELAND quotes the following extract from *Life in the Southern Isles* by Rev W WYATT GILL "On the Island of Vaur (southernmost of the Loyalty Group immediately to the east of New Caledonia) in the doubtful light, a native unhappily took a good sized shell-fish (*Conus textile*) and put it in his basket. He immediately felt a painful sensation running up his right arm to the shoulder. He went home. The pain increased until he writhed in agony. The body swelled to an enormous size and by daylight he was a corpse."

"(b) Early this year it was decided, at a conference held at Cairns, to form a Registry of Enquiries caused by Plants and Animals in Tropical Queensland, and accordingly questionnaires were forwarded to all the medical practitioners practising in North Queensland. The first case reported was by Dr T. B. CLOOSTER, then at Proserpine, to whom I am indebted for details of the fatal case here recorded

"C. H. G., a male age 27 years, whilst on a pleasure cruise landed at Hayman Island on 27th June, 1935 and picked up a live cone shell (since identified by Mr M. A. LOVEMAN, of the Queensland Museum, as *Conus geographus*). According to an eye-witness, it was gripped in the palm of one hand, with the open side downwards in contact with the skin, whilst with the other he proceeded to scrape with a knife, the epidermis, that is a thin outside covering the hard part of the shell. It was during this operation that he was stung in the palm of the hand. Just a small puncture mark was visible. Dr CLOOSTER did not see the patient until just before death, but the following details were obtained by him from the patient's mother who was present with him. Local symptoms of slight numbness started almost at once. There was no pain at any time. Ten minutes afterwards there was a feeling of stiffness about the lips. At 20 minutes the sight became blurred, with diplopia. At 30 minutes the legs were paralysed, and at 60 minutes unconsciousness appeared and deepened into coma.

"No effect was noticed upon the skin, lymphatic, alimentary or genito-urinary system. Just before death, the pulse became weak and rapid, with slow shallow respiration. Death took place 5 hours after the patient was stung.

"A postmortem examination showed that all the organs, heart, lungs, etc., were quite healthy.

"Mr J. B. HENDERSON, Government Analyst, reports that no poison was found in the stomach contents. The victim was, prior to the injury in perfect physical condition and in training for football.

"The symptoms resemble much those of curare poisoning as described in earlier reports. As usual, the puncture was in the hand and insignificant in size. The most striking difference was the entire absence of pain, although there was a feeling of stiffness. This is in contrast to Case 1 (ADAMS, 1850, *Conus asellus*) in which the pain was compared with the burning of phosphorus beneath the skin. Case 2 (CROOK and MAULE, 1874 *Conus textile*) in which severe pain persisted for some time. Case 3 (GARRETT 1878, *Conus tulipe*) in which there

was sharp pain, not unlike the sting of a wasp. Case 5 (HALLÉN 1901 also *Conus geographus*) in which the patient felt a sharp pointed thing which made her call out at once that she was bitten and poisoned. The victim of the fatal stinging by *Conus textile* (W. WYATT GILL, quoted by CLELAND 1915) immediately felt a painful sensation running up to the shoulder which increased until he writhed in agony.

The genus *Conus*, a gastropod mollusc, is quite a common one, there being nearly five hundred species, but reports of only five of them inflicting injury can be found in the literature. Although one at least, the dead shell of *Conus anemone* seldom seen alive, is frequently washed up on the beaches of Southern Australia, most other species are tropical. In the region of the Great Barrier Reef many species are common. Of those already mentioned, *Conus textile* is met with from time to time, but it is by no means either the largest or the most frequently met with, while *Conus geographus* is relatively rare and, according to HEDLEY so rare that few Europeans see it alive.

YASINO H. (1939) Fatal bite of *Conus geographus* *Venus* 9, pp 165-166 (Translation appearing in *Proc. Malac. Soc. London* (1940) 24, p 32.)

On 29th June 1935 a man, 32 years old, left home about 10 a.m. for bathing and shell collecting. Soon after he was infected by the bite of *Conus geographus*. He immediately felt great pain and scarcely managed to walk home. A doctor attended promptly the patient's temperature rose to about 36° C. (= 113° F) breathing became difficult and his finger tips were purple. He was soon unconscious and died about 3 to 4 hours after infection. The shell is stated to be about 13.5 mm. long perhaps this is a misprint for 13.5 cm. (or 135 mm.)

CLINCH, WILLIAM J & YOSHIO KONDO (1943) The poison cone shell. (Received for publication 23rd October 1942.) *Amer J trop Med.*, 23 pp 105-122.

During the past 100 years a few specific cases of death by the bite of a cone shell (*Conus*) have been published. The total list of known cases is not at all impressive and the danger from such a source may be very minor indeed, but it is present and constitutes a hazard and should not be overlooked by the shell collector in the tropics.

In these times when an ever increasing number of our armed forces are being sent to the tropics a surprising amount of interest has developed in the collecting of shells in places where they are come upon more or less casually as while swimming. This report is to focus attention on a potential hazard which might be encountered, especially in the South Seas, and to serve as a warning to those who would hardly expect a formidable danger in the form of a marine snail.

"The number of unrecorded deaths from this cause may be fairly extensive as the original bite may not have been noticed, particularly if the collector has suffered the usual cuts and scratches that one encounters while reef collecting especially among live coral.

The primary function of the bite of the cone shell is to paralyse its prey before feeding upon the immobilized victim—a condition strikingly parallel to the use of the poison apparatus in the rattlesnake. A secondary function, though to a lesser degree than in the rattlesnake, is that of protection.

"All cones, so far as our field experience would show are exceedingly sluggish and when taken from their environment remain almost completely inactive until they die. However it would be wise to consider all cone shells as potentially dangerous. We have made radula slides of several species and all are equipped with a rather formidable biting apparatus, though none, so far examined, equals in size that of *Conus striatus* figured in this report.

"In March, 1941 during the senior author's visit to the Hawaiian Islands, Mr. CLIFTON WEAVER, of Honolulu, collected a fine large specimen of *Conus striatus* off Rabbit Island, Oahu, in about three fathoms. The junior author dissected the specimen and made the drawings that accompany this paper.

"The mechanism of the actual delivery of the poison is still difficult to understand. The poison is apparently not delivered directly by means of the poison gland through the individual teeth from their base, but possibly after the wound is made by the tooth and then into the wounded surface, or perhaps through the hollow tooth after it is ruptured.

from the lingual membrane. It will appear that after the tooth has penetrated the victim a slight retraction would open or rupture the lower barb (Figs. 6 and 7) and thus open up the duct for the delivery of the poison. Thus may then open a channel through which the poison is subsequently ejected by pressure of the muscular proboscis, the entire process being very rapidly carried out."

Then follows a complete collection of original records from 1837 to 1939 and a description of the anatomy of *Conus striatus*. The poison gland usually known as the gland of Lefebvre is here called "Smaroth's Giftdrüse." The paper is illustrated by seven text-figures (drawings) and a plate illustrating the shells of *C. geographus*, *C. sulci*, *C. marmoratus*, *C. tulipa*, *C. textile* and *C. striatus*.

SUMMARY OF AUTHORS WHO HAVE ACTUALLY REPORTED CASES OF CONE STINGS,  
FATAL CASES BEING INDICATED BY AN ASTERISK.

*Conus sulci* Linn.

ADAMS, A. (1848)—Moluccas.

*Conus textile* Linn.

MACGILLIVRAY J. (1890)—New Hebrides.\*

CROSE and MARIE (1874)—New Caledonia.

MONTROUZIER, R. P. (1877)—New Hebrides.

WYATT GILL, Rev. W. (1894)—Loyalty Islands.

HEDLEY C. (1892)—New Guinea.

*Conus marmoratus* Linn.

MONTROUZIER, R. P. (1877)—New Hebrides.

*Conus tulipa* Linn.

CROSE, H. and MARIE, R. (1874)—New Caledonia.

GARRETT A. (1878)—Paumotu Islands.

BRAZIER (1884)—Isle J. C. Cox.

*Conus geographus* Linn.

HIND, Dr. BENJAMIN (1884)—New Britain.

HALL, Dr. A. HERBERT (1901)—Fiji.

SUGITANI, F. (1930)—Japan.

IREDALE, T. (1935)—Queensland.

CLOUTON, Dr. T. B. (1936)—Same case.

YASINO, H. (1939)—Japan.

HERMITTE, Dr. L. C. D. (1945)—Seychelles (1932)

# AN OUTBREAK OF *TRYPANOSOMA CRUZI* INFECTION IN INDIAN MONKEYS

BY

J. D. FULTON\*

AND

C. V. HARRISON

*Liverpool School of Tropical Medicine and the Department of Pathology  
Liverpool University*

*Trypanosoma cruzi* is generally considered to be a parasite of the New World. However, MALAMOS (1935) found a trypanosome in the blood of a number of *Macacus cynomolgus* monkeys imported from Java which had been only a short time in Hamburg. He showed from its morphological characters, method of reproduction in mouse tissues, pathological anatomy and transmission by *Triatoma infestans* that the parasite was *T. cruzi*. None of the monkeys naturally infected with these trypanosomes showed clinical signs of illness, and the infection was mild in character periods in which trypanosomes in the blood were scanty being followed by negative periods. Sub-inoculation to other monkeys gave rise to infection after a long incubation period. The course of the disease was unaltered by splenectomy except that, if carried out during a latent phase, parasites reappeared in the blood. Other laboratory animals were infected and the morphology of the trypanosome in passage animals was identical with that in the naturally infected monkeys. He suggests that *T. cruzi* infection is not confined to the New World.

Trypanosomes have also been found in the blood of Asiatic monkeys by other authors. BRUMPT (1909a) found trypanosomes in the blood of a young

\* Member of the Scientific Staff of the Medical Research Council.

on this date did not seem well, and remained at the bottom of his cage with head down. Trypanosomes were found daily in the blood till 23.6.44. The animal, then moribund, was killed with ether. Mice were inoculated with its blood and became infected with trypanosomes. Postmortem examination revealed nothing abnormal in the organs. Sections of liver, spleen, kidney and heart were made. The *heart* showed no lesion, in numerous sections. The *liver* showed malabar pigment and some fatty change, but no other lesions. In spite of the absence of any suggestive lesions, the sections were examined for parasites, but none were found. The *kidney* showed one small focus of cellular infiltration similar to that seen in MR28 situated at the junction of cortex and medulla. We failed to find parasites in it. The *spleen* contained considerable malarial pigment. In many of the Malpighian bodies there was focal hyperplasia of the reticulum cells of the germ centre. Leishmania stages were demonstrated in these.

MR41 This animal was inoculated with blood from MR34 on 12.1.44. After several attacks of malaria, which were treated with drug, its blood was used to infect MR42 on 21.2.44. It was splenectomized on 24.3.44 and killed for another purpose on 19.5.44. It remained well throughout, and trypanosomes were never found in its blood.

MR42 was infected with malarial blood from MR41 on 21.2.44. It had several attacks of malaria from which it recovered with treatment. Although its blood was examined regularly for trypanosomes for several months, none were discovered till 18.9.44 when only one trypanosome was found in 200 microscopic fields. They remained scanty till 1.10.44 when the animal for the first time appeared to be ill and remained lying down. On 2.10.44 it had a convulsive attack with twitching of arms and legs. Its face was swollen, and it cried as if in pain. Between the convulsive attacks it was inco-ordinated and clung to the bars of the cage while its face and mouth twitched continuously. As in some of the other infected animals, there was a mucous discharge from the nares. On 3.10.44 the convulsive attacks continued, sometimes beginning while food was being taken, so the animal was killed with chloroform. Postmortem examination revealed subcutaneous oedema of the face as the only abnormality. The animal was well nourished. Examination of the gut contents for *E. histolytica* was negative. The rectal temperature just before death was 100.4 F. Sections were made of various tissues. The *spleen* showed lesions exactly similar to those of MR40 and leishmania stages were demonstrated. The *bone marrow* (femur) contained several foci of macrophage infiltration up to 200  $\mu$  diameter. Leishmania stages were found (Fig. 5). The *heart* showed a few small foci of macrophage infiltration, all less than 100  $\mu$  diameter both in the subpericardial connective tissue and in the myocardium. Leishmania stages were found in both sites (Figs. 3 and 4). The *liver* contained considerable malarial pigment, and showed a few small foci of macrophage infiltration, but we were unable to demonstrate any parasites. In addition, the following tissues were examined without any lesion being found: Pancreas, kidney, brain, lung, adrenal and voluntary muscle.

MR43 was inoculated with malarial blood from MR42 on 22.3.44. After drug treatment, parasites were last seen on 20.4.44. On 16.5.44 the animal was splenectomized, and appeared to have been cured of malaria, as there was no recurrence of infection. On 13.8.44 after repeated examinations over a period of many weeks, a scanty infection with trypanosomes was noted in a fresh blood preparation. On 18.8.44 the animal collapsed and died suddenly while under observation, without any previous suggestion of illness. Trypanosomes had increased in numbers to three per field on this date. Postmortem revealed nothing abnormal to the naked eye, and the animal appeared to be well nourished. *E. histolytica* was not found in the gut contents. Sections were made of different organs. In the *heart* three small foci of mononuclear infiltration were seen, two in the muscle and one in the pericardium. No parasites were found. The *liver* showed diffuse infiltration of the portal tracts, but there were none of the focal lesions seen in MR26 and MR42. No parasites were found. The *bone marrow* showed a slight mononuclear infiltration into the plasma-sinoid and into the Virchow Robin spaces. No parasites could be found. The *lung* and *kidney* showed no lesion.

MR44 was a stock animal which had never been used for any purpose. On 15.8.44 scanty trypanosomes were present in its peripheral blood. They were last seen on 23.8.44. The blood was regularly examined till 14.12.44 with negative results, and the animal was killed on that date having remained in good health. Apart from sclerosed glands

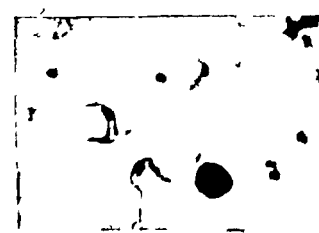
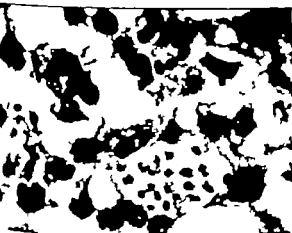
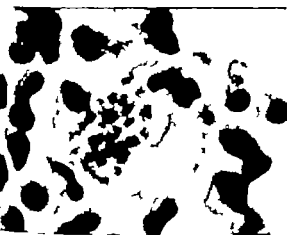
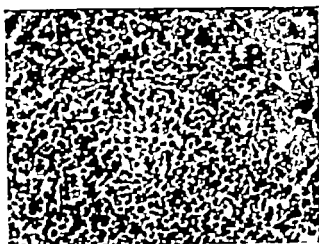


FIG. 1—Blood films of MR31 showing *L. crudi* ( $\times 1400$ )

FIG. 2—Blood films of mouse infected from MR41 showing *L. crudi* ( $\times 1400$ )

FIG. 3—Pericardium of MR42 showing leishmaniasis surrounded by a mononuclear infiltration ( $\times 1700$ )

FIG. 4—Myocardium of MR42 showing a focus of mononuclear infiltration ( $\times 1400$ )

FIG. 5—Bone marrow of MR42 showing leishmaniasis ( $\times 1800$ )

FIG. 6—Liver of MR20 showing focus of mononuclear infiltration ( $\times 220$ )





in the skull and groin, nothing abnormal was noted postmortem. Sections were made of various organs. No lesion or parasite was found in the heart or liver. In the kidney there was one minute focus of mononuclear infiltration, but no parasite was found. The lung showed some oedema, but was otherwise healthy. The spleen showed hyperplasia of the germ centres of the Malpighian bodies, similar to that seen in MR40 and MR42, but we were unable to demonstrate parasites. The bone marrow showed one minute focus of mononuclear infiltration similar to that seen in MR42, but we failed to find any parasites. The axillary lymph glands showed a simple inflammatory hyperplasia. No parasites were found.

### SOURCE OF INFECTION

The source of infection in these animals was naturally our first concern. Had infection occurred before arrival or subsequently? MR26 and 31 had been in this laboratory approximately 2 years before trypanosomes were found in their blood. They were housed in a separate room from mice in which a laboratory strain of *T. cruzi* is maintained. From the above histories it appears that MR37 may have been infected with trypanosomes and was the first of the monkeys to die. As a trypanosome infection was not then suspected, only stained preparations of its blood were examined and parasites could easily have been missed if numbers were small. It is noteworthy that MR26 and MR31 both inoculated with its blood on the same day, had trypanosomes in their blood within 2 days of one another about 6 weeks later. MR31 was the second animal to die. Whether MR34, which was the original source of the malarial infection for the monkeys concerned was infected with *T. cruzi* as well cannot be stated, especially as MR44 which had never been inoculated, harboured trypanosomes which could only be found in the blood over a short period. The effect of splenectomy to which some of the animals were subjected is of doubtful significance in causing the trypanosomes to appear in the peripheral blood in numbers sufficient for observation. MALAMOS (1935) noted however in the case of *M. cynomolgus* infected with *T. cruzi* that if splenectomy were carried out during a latent phase, trypanosomes did reappear in the blood.

It seemed possible that the infection might have been conveyed from infected mice by ecto-parasites generally to be found in a laboratory either directly or through faeces. A search was therefore made for bed bugs which we knew were sometimes imported in sawdust. In all, thirteen bugs were obtained from wooden boxes with sawdust bedding used to house stock rats, but were never found in the metal monkey cages. The alimentary canals of these bugs were examined for flagellates and suspensions of them were injected into mice, all with negative results. BRUMPT (1912) allowed *Cimex lectularius* to feed on animals infected with *T. cruzi* in the laboratory and the bugs became infected in 100 per cent. of cases. The faeces of these bugs containing flagellates proved infective to young rats on inoculation. BLACKLOCK (1914) also showed that *T. cruzi* could live and multiply in *Cimex lectularius* for long periods, and that the parasites in the bug are infective for laboratory animals. Transmission

of the disease to healthy animals by feeding infected bugs on them was of very rare occurrence.

Our monkeys were themselves infested with lice (*Pediculus longicaps*), which were removed by means of a fine comb. Several dozens were ground up at a time in saline on different occasions and the suspensions then examined for flagellates, but none were found. Mice were regularly injected intraperitoneally with such suspensions but no infection with *T. cruzi* resulted.

Fleas were never found on the monkeys.

The blood syringes used for inoculating monkeys were kept for that purpose alone, and it was not possible that infection from outside sources could have been caused by this means.

When these investigations had been completed, information was obtained that the ship in which the monkeys had been transported had called at a number of ports in South America and had passed through the Panama Canal, which is of possible significance regarding the source of infection.

#### IDENTIFICATION OF THE PARASITE.

The trypanosome found in the blood of the above monkeys was morphologically indistinguishable from a strain of *T. cruzi* maintained in mice in this laboratory. The large blepharoplast and other features as seen in stained films, and the peculiar lively movement of the trypanosomes in wet preparations of blood were characteristic. Mice were inoculated with the blood of each infected monkey and the course of the resulting infection in these animals was followed at the same time as that produced by our stock strain of *T. cruzi*, also maintained in mice without any differences being noted. The morphology of the monkey parasite was unaltered by passage in mice. The oedema produced in some mice by our laboratory strain of *T. cruzi* (COLLIER, FULTON and LIXER, 1942) also occurred in some mice inoculated with the monkey trypanosome. This oedema has never been noted by us in mice infected with any other species of trypanosome. Camera lucida drawings were made of the trypanosomes from stained films of monkey blood, from the blood of mice infected from monkeys, and from mice infected with our normal strain of *T. cruzi*. The average lengths of the trypanosomes were respectively 18.3  $\mu$ , 17.7  $\mu$  and 18.5  $\mu$ . WENTON (1926) gives the length of *T. cruzi* as approximately 20  $\mu$ . The occurrence of broad and slender forms was noted in all three types of infection. The blood of mice infected from monkeys was cultured in KEELER's medium (1936) at the same time as that of mice infected with our laboratory strain and similar results were obtained. Leishmania forms growing in clusters, as well as flagellate forms, were found in each case. Leishmania forms were also present in the tissues of mice infected from monkeys, and in the liver, spleen, heart, pericardium and bone marrow of the infected monkeys themselves on different occasions.

From the above findings, we conclude that the parasite was *T. cruzi*.

## DISCUSSION

The source of infection in these monkeys is still not beyond all doubt. We have, however, been keeping batches of *M. rhesus* for a number of years under similar conditions and have not noted any similar infection with trypanosomes, nor have any other monkeys died under similar circumstances. Some of these animals sickened and were ill for some days before death, but in the case of MR37 and MR43 death was sudden and no definite reason can be given to account for it. MR43, for example, collapsed in the middle of a meal without previous signs of illness, and scanty trypanosomes had been present in its blood for only a few days. This is in marked contrast to the experience of MALAMOS (1935) with *cynomolgus* monkeys. He never noted any clinical signs of illness in these animals naturally infected with *T. cruzi*. DAVIS (1943) had two deaths in a batch of ten *M. rhesus* monkeys which he infected with *T. cruzi* in the laboratory. Fewer trypanosomes were found in the blood than was the case with our animals, and he was not certain that the trypanosome infection was the cause of death. Whether the previous bouts of malaria in the present instance contributed to the fatal issue is not known. It may be noteworthy that MR44 which was most carefully observed over a period of 6 months and had never been infected with malaria, had a scanty trypanosome infection which lasted only 13 days. This animal remained well and free from parasites for 4 months thereafter, and when it was killed sections of various tissues failed to reveal the presence of leishmania forms.

## SUMMARY

Of a batch of ten *Macacus rhesus* monkeys received from India, the blood of four was later found to harbour trypanosomes. Two other monkeys of a previous batch, inoculated from an animal which subsequently died under suspicious circumstances before a trypanosome infection was suspected, also became infected.

The infecting trypanosome was shown by morphological and cultural characters, as well as by its method of reproduction in tissues to be indistinguishable from *Trypanosoma cruzi*.

Transmission from one monkey to another by blood inoculations was possible in some cases, but one infected animal had never been inoculated.

The source of the infection has not been definitely traced. It is known that the ship transporting the monkeys called at ports where *T. cruzi* infections occur in man, insects and animal carriers and infection might thus have occurred during the journey. We cannot, however, completely exclude a laboratory infection.

The possibility of *T. cruzi* infection should be borne in mind when working with rhesus monkeys.

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## REPORT OF A CASE OF MULTIPLE IDIOPATHIC HAEMORRHAGIC SARCOMA OCCURRING IN A WEST AFRICAN NATIVE.

BY

WALLACE DENNISON MAJOR, R.A.M.C.

AND

WINSTON EVANS MAJOR, R.A.M.C.

Since KAPOSI (1872) first described this condition a large number of cases have been recorded in the literature. The disease is met most frequently among the labouring classes of Russia, Poland and Italy and occurs not infrequently among the industrial classes of most large cities. This communication is of interest in that it records an instance of the condition in an African.

### CASE REPORT

The patient, a Nigerian soldier aged 30 years, was admitted to hospital on 26.2.43 as a case of Madura foot. His right foot was swollen as in maduro-mycosis and presented on the dorsum fifty raised nodules varying in size from  $\frac{1}{8}$  to  $\frac{1}{4}$  inch in diameter (Fig. 1). The right leg showed pitting oedema.

The condition had existed for over 3 months but the patient felt well and stated that he had lost no weight.

The nodules felt fibrous and appeared to be fibromata. Scrapings showed neither sulphur nor black granules. No organisms were seen in smears and none were isolated on culture. A typical nodule was removed for histological examination.

No glands were palpable in the popliteal spaces and the inguinal glands were within normal limits for an African and were approximately the same on both sides. There were no typical yaws scars and there was no evidence of syphilis. The Kahn test was negative and remained so after a provocative dose of N.A.B.

Blood examination revealed no abnormality and malaria and filaria parasites were not found in thick films.

No abnormality was detected on examination of heart, lungs, abdomen, faeces or urine.

On 5.11.43 the number of nodules had increased to over 100. There was no evidence of secondary involvement of the regional lymph glands and the patient still felt quite well.

### Histological Report

A circumscribed tumour characterized by a proliferation of atypical spindle cells arranged for the most part in whorls and interlacing bundles giving the impression of a sarcomatous neoplastic process (Fig 2). The nuclei of the spindle cells are vesicular and pale staining showing some margination of the chromatin (Fig 2). hyperchromatic nuclei and mitotic figures are not present.

The spindle cell proliferation appears to be related to small angiomatous areas with swelling of the endothelium and proliferation of the perivascular cells.

There is much infiltration by lymphocytes and plasma cells, and haemorrhage has occurred into the tissue spaces. There is very little pigmentation. Hyperkeratosis is a marked feature and there is some fibrosis of the derma.

Fig 2 is a low power view showing the cellular arrangement of the tumour and Fig 3 is a high power view of a region of Fig 2 illustrating the detail of the atypical spindle cells and their relation to an angiomatous area.

### COMMENTARY

The occurrence of Kaposi's disease in an African presents an interesting problem for diagnosis and includes the consideration of the following diseases: (a) multiple fibromatosis, (b) maduromycosis, (c) yaws, (d) syphilis, and (e) elephantiasis with upset in skin nutrition. As some of these conditions may co-exist, differentiation may well be rendered difficult more especially if the histological picture of the tumour is predominately inflammatory in type consisting chiefly of perivascular oedema and infiltration by lymphocytes and plasma cells. In the case described the lesions were essentially of the proliferative type composed of atypical spindle cells of uncertain origin giving the impression of a neoplastic process. Their relationship with angiomatous areas would suggest a probable origin from vascular endothelium.

Neither our clinical nor histological observations suggest any new explanation for the condition. They merely emphasize its typical mixture of inflammatory and neoplastic processes and its specific angiomatous features. Its occurrence in a young adult is uncommon and the degree of hyperkeratosis, plasma cell infiltration, and the small amount of pigmentation are the unusual characteristics.

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FIG 1

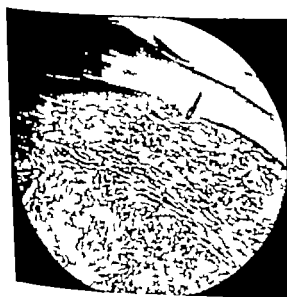


FIG. 2.—Low power view of tumour

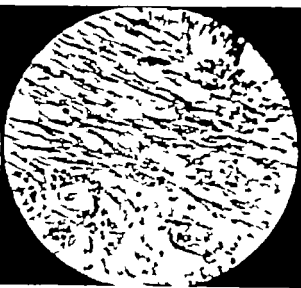


FIG 3—High power view





## SOME OBSERVATIONS ON *SALMONELLA* STRAINS IN DOGS MICE AND TICKS

BY

RUDOLPH REITLER, M.D (VIENNA),

AND

RUDOLPH MENZEL, M.D (VIENNA)\*

*From the Government Laboratory Haifa, and the Canine Research Institute  
Kiryath Motzkin.*

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During the last few years a peculiar canine disease has been observed mainly amongst dogs living in large groups (kennels, or dogs belonging to communal settlements) and particularly in localities where infestation with ticks was rife. The disease somewhat resembles distemper or Stuttgart disease, but differs from both in many respects. Our attention was drawn to it in 1939 when one of us (R. M.) introduced the disease unintentionally into his own kennel by accommodating ten semi wild pariah dogs which were intended to be used for breeding experiments. Out of these ten animals nine died in a short time and since then the disease continued endemically amongst the European dogs (boxers and alsatians) until it came to a standstill in the winter 1942-43. Its peak was reached in the summer of 1942 when tick infestation was considerably higher than in other summers.

\* We beg to express our thanks to Dr MACQUEEN Director Medical Services of Palestine, and to Dr HENNESSY Deputy Director (Laboratories) for permission to publish this article.

The incubation period of the disease appears to be about 2 weeks. Its duration and symptoms vary considerably from a hyperacute course lasting less than a week to a chronic condition of 2 years' duration with temporary improvements followed by relapses.

The fulminating cases give the impression of a septicaemia with high fever, tonsillitis, generalized enlargement of lymphatic glands and enlargement of the spleen, diarrhoea, complete anorexia and extreme apathy.

The chronic cases may start in the same way—more frequently however an insidious onset is observed with slight diarrhoea and glandular swellings. The disease develops in such cases slowly, lasting on an average about 2 months and evolving into a much more varied picture with roseolar spots on the skin, cough, sometimes pneumonia, rhinitis, epistaxis and encephalitic or neuritic symptoms (isolated or generalized paralysis of muscle groups). As the disease progresses the glandular swelling and spleen enlargement become more marked, the dogs are very anaemic and eye-complications such as keratitis, scleritis and iritis with depigmentation are often observed. In contrast to the initial anorexia the dogs become voracious but in spite of that they get progressively weaker and eventually die from emaciation perhaps partly due to anaemia, or during an attack of pneumonia. The blood picture shows a normal number of leucocytes but a more or less severe hypochromic anaemia without signs of regeneration.

Sudden spontaneous recovery occurs sometimes mainly in young animals, but as a rule convalescence is very protracted in those animals which recover at all. In young animals enamel defects of the teeth occur frequently after recovery as seen after distemper. Bitches contracting the disease during pregnancy invariably abort and if they are mated during convalescence relapses occur frequently leading also to abortion. There is hardly any difference in susceptibility between young and adult dogs. Therapeutically no drug has proved to be efficient. Sulphanilamides had, if any, only an unfavourable effect.

Other diseases, mainly helminthic infestation, very frequently complicate the condition and it is difficult to say how far they contribute to the severity and the duration of the disease. In particular infestation with *Deosomus* seems to have a very unhappy influence and considerable improvement in chronic cases has sometimes been achieved by anthelmintic treatment. Thus, it may be that very long lasting conditions are, in the later stages, caused by the complicating factor alone while the primary infection may have already subsided long ago.

Occasional concomitant infections with *Rickettsia celsi* (DOMATION and LANTOCCARI, 1935), which is transmitted by ticks, can also not be ruled out with certainty although blood smears and glandular punctures never revealed the presence of rickettsiae nor has this particular rickettsial infection ever been observed in Palestine.

In view of the rather ill-defined clinical picture it is hardly possible at present to give any mortality rate. Some cases of different aetiology may have been wrongly attributed to this disease and some mild cases may have been missed. As we have now simple diagnostic means at our disposal, namely agglutination and culture, it will be possible in future to assess the death-rate more correctly—for the time being, it may be estimated at about 40 per cent.

As frequently repeated parasitological examinations of blood, glandular and spleen punctures invariably yielded negative results for all protozoal diseases we tried to inoculate subcutaneously 10 c.c. citrated blood from a severely ill bitch ("Lilith") into a guineapig. At the same time another part of the blood was cultured in glucose broth.

The broth culture was turbid after 24 hours and by further examination the cultured organism was revealed to be a *Salmonella*. The strain was forwarded to the Representative of the *Salmonella* Committee of the League of Nations Department of Hygiene, Prof. A. KLOPSTOCK, Tel Aviv, who kindly examined its antigenic structure and determined it as VI, XIV XXIV r1, 7. The O antigens and the unspecific phase of H are, therefore, identical with *S. carrau* but our strain differs from it by the specific H antigen which is in *S. carrau* "y". It differs also from *S. abortus canis* (GARD 1938) whose antigen formula is IV, V XII-b z, z.

The inoculated guineapig developed a cellulitis at the site of injection and died following progressive emaciation on the 8th day after inoculation. On postmortem examination the spleen was found considerably enlarged, the pericardial cavity filled with turbid fluid and the heart muscle gray and very friable. Microscopically, the spleen showed the usual picture of an acute infection and the heart a suppurative pericarditis extending to the external layers of the heart muscle. Cultures from spleen and pericardial fluid yielded the same *Salmonella* strain as found in the blood culture of the bitch.

Meanwhile, the epidemic began to subside and we only had the opportunity to examine by blood culture three rather severely ill dogs. Of these two were negative but one bitch yielded again the strain "Lilith". Stool examinations could be carried out only for a few chronically ill dogs—all proved to be negative.

At the same time (Autumn, 1942) an epidemic with high mortality broke out amongst the white mice in our Haifa Laboratory as well as in the Bacteriological Laboratory of the Hebrew University Jerusalem. In both instances the causative organism was found to be a *Salmonella* identical in its antigenic structure with our strain "Lilith" (information about the Jerusalem outbreak obtained by kindness of Dr. OLITZKY, Department of Hygiene, Hebrew University). In the Haifa outbreak a spread of infection from the canine specimens was still conceivable although highly improbable but this explanation cannot be applied to the Jerusalem epizootic as this laboratory had nothing to do with the disease in dogs and had no connection whatsoever with the Haifa Laboratory. We cannot but suppose that at this time the strain was particularly common in the susceptible animals of this country. On the other hand, it seems to be non-pathogenic for man. We included the strain for a while in our set of strains for diagnostic routine agglutinations and had not a single positive result in more than a hundred human sera.

The fact that an outbreak of the disease coincided with an abnormally high tick infestation led us to the examination of these parasites. One hundred and six ticks (*Rhipicephalus sanguineus*) were collected from chronically ill or convalescent dogs, dipped into tincture of iodine, dried, washed with 60 per cent alcohol and dried again. The abdominal cavity was opened with sterile scissors and the whole tick put into ox bile broth.

In one adult female tick the culture was positive. But the salmonella recovered from her had the antigenic formula IX-g,p,m—that is to say it was identical with *S. enteritidis* and totally different from our strain "Lilith." The bitch from which this tick had been taken was just recovering from the disease. Her blood culture was negative but her serum agglutinated the strain from the tick to a dilution of 1:2,560 while the agglutination with strain "Lilith" was negative. Thus, it became obvious that we were confronted with two different endemic salmonella infections in the same locality. But just as we had decided to disentangle them by agglutination tests the scientific ambition of a kennel maid contributed still more to the confusion. While taking a walk in the fields she found a dying hedgehog and, aware of our investigations, she picked eight ticks from it and brought them for examination. Unfortunately the hedgehog was left behind. In one female tick an immotile *Salmonella* strain was found—this time with the antigenic formula XVII— that is to say an immotile *S. kerkera* strain.

These findings are of course no strict proof that ticks act as transmitters in salmonella infections and in the two mouse epizootics any part played by ticks can definitely be excluded. But their role as occasional transmitters is very probable in the light of experiments carried out by PARKER and STEINHAUS (1943) who succeeded in transmitting *S. enteritidis* to guinea-pigs by the tick *Dermacentor Andersoni*. The salmonella is harboured by these ticks for as long as 5 weeks and may be passed through the egg to the larva.

With our three strains we have carried out agglutination tests with sera of twenty-seven supposed survivors of the disease and of four healthy contacts. None of the dogs' sera agglutinated the *kerke* strain from the hedgehog. The agglutinations with the other two strains as far as they were sufficiently high to be considered as significant (above 1:80) are summarized in the following table—

Agglutination Positive with	Name.	Sex.	Total Duration of Disease.	Interval between Recovery and Agglutination.	Titre.
<i>Salmonella enteritidis</i>	Bilba	F	3 months	3 days	~160
	Ragan	M.	2 "	3 months	160
	Ranon	M.	" "	2 "	160
Strain "Lilith"	Doron	M.	2 months	6 weeks	640
	Elitz	M.	6 days	8 months	250
	Shoddah	F	1 month	9 "	250
	Zails	F	2 weeks	2 days	160
	Douglas	M.	14 months	Chronically ill died later	160
	Lilly	F	—	Healthy Contact with Douglas	250

Thus, we see that out of twenty-seven dogs which had been ill, eight gave a significantly high agglutination three with *S. enteritidis* and five with the strain 'Lilith.' The other nineteen either did not agglutinate at all or did so with a titre of or below 1/80. But it has to be noted that the sera of both dogs from which we obtained the positive blood cultures agglutinated their own strain also not more than 1/80 at the height of the disease and both died soon afterwards. Out of the four healthy contacts three did not agglutinate at all, while one bitch, probably after having passed through a subclinical infection showed a higher titre than the frankly ill dog with which she had been in contact.

#### SUMMARY

1. *Salmonella* infections in dogs have been observed in Palestine partly with *S. enteritidis* and partly with a new strain with the antigenic formula VI XIV XXIV-r-1/7. This latter strain was also responsible for two epizootics in laboratory mice.

2. It is very likely that ticks act occasionally as transmitters of salmonellas as *S. enteritidis* was found in a tick from a dog and *Salmonella kirkee* (var. *immobilis*) in a tick from a hedgehog.

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# OBSERVATIONS ON AN ANTBEAR (*ORYCTEROPUS AFER*) IN RELATION TO INFECTION WITH *TRYPANOSOMA* *RHODESIENSE*

BY

E. BURTT PH.D., D.I.C.

*Entomologist Sleeping Sickness Research Station, Tinde Tanganyika Territory*

VANDERPLANK (1941) was the first observer to record the experimental infection of an antbear with *Trypanosoma rhodesiense*. A single animal was used and its mean body temperature prior to infection was 97° F (from ten observations). After infection with *T. rhodesiense*, 2 per cent. of the 400 *Glossina morsitans* fed on it developed salivary gland infections. Two rats bitten by one of these infected flies lived 16 and 20 days respectively. Records at Tinde laboratory show that the specimen studied by VANDERPLANK lived 108 days after infection, death being apparently due to trypanosomiasis.

During November 1941, another antbear about half grown, was brought to the laboratory. It measured 3 feet from head to tip of tail. It thrived on milk and, later, termites were added to the diet. After 2 months early morning rectal temperature readings were taken for a fortnight. At first the animal resisted vigorously but by the 2nd day it was more docile and soon became accustomed to this procedure. Temperature readings were then discontinued until 4 days before its infection on 5.5.42 by fly C V 19 which had been isolated from a Thomson's gazelle. Temperature readings were continued daily until the animal's death on 26.6.42. It lived 51 days from the date of infection. Three batches of flies each comprising 132 individuals were applied to the antbear. Two of these gave transmissions to clean animals, four infective flies



being found by isolation on rats. In all thirty rats were bitten by these flies. The data relating to temperature, the course of the infection, transmissibility in flies and longevity of infected rats are given below

### I—TEMPERATURE.

#### (a) Before infection

1942. Jan 20	97.6° F	Indication of progressive decrease of night
21	95.6° F	
22	95.4° F	
23	95.0° F	
24	95.2° F	
26	95.0° F	
27	95.0° F	
28	95.0° F	Two separate observations and observers.
29	95.0° F	"
30	95.0° F	"
31	95.0° F	
Feb 2	95.0° F	Two observers and the thermometer was again checked on the writer
May 1	95.0° F	
2	95.0° F	
3	95.0° F	
4	95.0° F	

#### (b) After infection

Fifty three observations were taken between 5.5.42 and 26.6.42. The temperature was 95.0° F throughout with a maximum variation of + 0.2° F on 2 widely separated days.

### II—THE COURSE OF THE INFECTION

5.5.42.—Fly C V 19 ex Thomson's gazelle 31 bit twice without feeding. The blood was examined on 10th, 11th and 12th May but was negative in 200 fields of a stained thick film.

13.5.42.—Blood + 2/250 fields, stained thick film (incubation period of *T. rhodesiense* in the Antbear 8 days).

14.5.42.—Blood + 12/15 fields, stained thick film.

16.5.42.—Blood + 13/50 fields, stained thick film.

From 17th to 26th May the blood was negative in seven examinations. On 19th May two rats were inoculated each with 1 c.c. blood from the jugular vein. They remained negative. On 26th May two further rats were similarly inoculated. Both were infected (life 55 and 62 days respectively).

29.5 42.—Blood + 18/1 fields, stained thick film

Two rats were inoculated each with 1 c.c. blood from jugular vein Both were infected (life 70 and 97 days respectively)

30 to 31.5 42.—Blood — 0/200 fields, stained thick film

1 6 42.—Blood + 1/200 fields, stained thick film

2 to 12.6 42.—Blood — 0/200 fields, stained thick film (four examinations)

15.6 42.—Blood + 10/1 fields stained thick film

16 to 26 6 42.—Blood — 0/200 fields, stained thick film (eleven examinations)

The anthrax appeared unwell on 9th May but recovered next day apart from this instance, it remained in good condition until very shortly before its death when it deteriorated rapidly and showed considerable emaciation.

26 6 42.—Died (life 51 days)

### III.—INFECTION OF *G. morionis*.

Experiment.	Transmission	Flies.				
		Date first applied to host	Number used.	Number examined.	Number + ve	Percentage infected.
1	+	14.5.42	132	52	3	5.8
2	+	17.5.42	132	42	1	2.4
3	Nul	16.6.42	132	18	0	0

### IV.—THE LONGEVITY AND INCUBATION PERIOD OF RATS INFECTED BY FLIES ISOLATED FROM THE ANTHRAX.

Fly	Number of rats.	Life of rats in days.	Incubation period of rats in days.
G.Z.24	7	36 36 39 36 42 38, 50	6 5, 5, 5 6, 5, 6
G.Z.39	12	58, 36, 39 34 58 31 36 62, 42, 50 44, 40	6 9 11 5 6 5 6, 7 5 5 5, 5
G.Z.40	2	35, 54	6 6
H.A.27	0	28 3., 37 39 35, 48 42, 3* 34	6, 7 6 5 5 7 5 5 6
Total	30	Average 40.5 days	Average: 5.9 days

## COMMENTS.

VANDERPLANK'S record for an antbear was given along with data which pointed to a possible relationship between normal temperature of host, transmissibility in flies and virulence of the trypanosomes as manifested in rats (*G. morrisoni* and *T. rhodesiense*). His suggestion was that the lower the normal temperature of the host prior to infection, the higher was the subsequent transmissibility of the trypanosomes in flies and the shorter was the length of life of rats bitten by infected flies (i.e., the greater was the virulence of the trypanosomes). His data for antbear did not entirely give support to this scheme. The temperature was 97° F., salivary gland infected flies 2 per cent. and infected rats lived an average of 18 days. This short length of life of rats conformed with his suggestion but the low transmissibility found in flies did not.

In the present investigation the temperature of the antbear was 95° F., 1.5 per cent. of flies had infected salivary glands (in two experiments which gave transmissions) while rats bitten by infected flies lived an average of 40.5 days. These results thus agree with those of VANDERPLANK in the low rate of infection found in the flies, but do not support his suggestion that transmissibility and virulence of a strain of *T. rhodesiense* may be enhanced by passing through animals with a low mean body temperature.

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## CEREBRAL LESIONS PRODUCED IN HEALTHY DOGS BY THE INTRAVENOUS INJECTION OF 4 4-DIAMIDINO STILBENE

BY

E. G. OASTLER, LIEUT-COLONEL, R.A.M.C.,  
AND

H. K. FIDLER MAJOR, R.A.M.C.  
(From a British General Hospital, M.E.F.)

The cerebral lesions to be described in this paper were the result of the injection of freshly prepared solution of 4 4'-diamidino stilbene. They were found during the course of an investigation which was originally carried out because of the death of two patients who had been treated with the drug.

Two cases of Sudanese kala azar were treated in our hospital in September 1941 with 4 4'-diamidino stilbene, totals of 3.15 grammes and 3.05 grammes were given respectively by intravenous injection over a period of 3 weeks. The drug was sent to us from the Stock Laboratories, Khartoum already prepared as a 1 per cent. aqueous solution, and at the time of its administration was from 1 to 4 weeks old. Both patients were apparently cured of the disease but died late in convalescence. One patient died of acute haemorrhagic pancreatitis 3 weeks after finishing the course of injections and had minimal central necrotic lesions in the liver and tubular nephritis. The other patient died of terminal hypostatic pneumonia 7 weeks after the last injection. Postmortem examination showed tubular nephritis, early cirrhosis of the liver and chronic interstitial pancreatitis.

These lesions suggested a toxicity of the drug and in order to investigate this eight healthy dogs were given daily intravenous injections of the same stock solution. The daily dosage was rather less per kg. of body weight than that given to the patients. The lowest average daily dose was 0.8 mg. per kg. of body weight and the highest was 2.1 mg. per kg. of body weight. Only one animal survived the course of twenty-one injections. Five died at varying intervals during the course and had tubular nephritis and minimal central lobular necrotic lesions of the liver. Two died suddenly immediately after the injections on the 4th and 6th days respectively and did not show any such pathological changes. The animal that survived the course and was killed 6 weeks later showed a healing stage of both the liver and the kidney lesions.

The drug used for the patients had been made up in solution for some time and when used for the dogs it was from 4 to 6 months older. During the interval no precautions had been taken to protect the solution from the light. The question arose as to whether these lesions might have been due to a toxic effect produced by some change in the drug when used as an old solution. Therefore a series of ten dogs was injected with a fresh solution of the drug made up immediately before each injection. Using this freshly prepared solution no liver or kidney lesions were produced.

completely conscious. (Fig. 1) It died next day. Postmortem examination revealed large regions of softening and haemorrhage in both optic thalami, the mid brain and the cerebellar peduncles. Macroscopically the lesions were similar to those described in Dog 4 with the addition of a mild and subacute inflammatory infiltration of the basal meninges. This appeared to be a direct spread from the lesion in the cerebellar peduncles.

Dog 6 15 kg. A total dose of 45 $\frac{1}{2}$  mg. was given in twenty-one injections making an average daily dose of 1.45 mg. per kg. body weight. After the first injection the animal immediately urinated and defaecated, and appeared ataxic. Similar but less marked ataxia was noticed after the second injection. On the 8th day in addition to marked ataxia, a wasting stamping gait was present, which was more marked in the forelegs. This remained constant for 4 days and then gradually disappeared during the succeeding week. On several occasions there was swelling of the eyelids coming on immediately after the injection and lasting for several hours. The animal appeared healthy when it was killed on the 42nd day (3 weeks after completion of the course). Postmortem examination showed in the anterior part of each caudate nucleus a small haemorrhagic cystic lesion measuring about 2 mm. in diameter. No other brain lesions were found. Microscopically the caudate lesions consisted of a central collection of well preserved erythrocytes surrounded by a narrow zone in which the myelin was vacuolated and infiltrated with large cells containing brown pigment. There was some perivascular cuffing with small round cells in the region near the lesion and also an apparent increase in glial cells in the zone of demarcation. (Fig. 4)

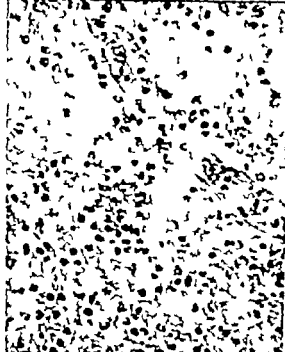
Dog 7 14.1 kg. A total dose of 60 mg. was given in four injections making an average daily dose of 1.46 mg. per kg. of body weight. Immediately after the first injection the dog became excited and barked wildly. After the next three injections it whimpered and appeared ill. On the 5th day it was unconscious. Its legs were extended and spastic and its head retracted. It swallowed frequently but there was no salivation. It died later in the day. Postmortem examination showed a greyish red region of softening and haemorrhage in the roof of the fourth ventricle. This extended into the cerebellum for a depth of 1 cm. A similar region of softening and haemorrhage was found in the cerebral cortex of the left occipital lobe. Microscopic examination showed myelin degeneration and infiltration with erythrocytes. In some places this was most noticeable in close relation to small blood vessels. The walls of some blood vessels were infiltrated with polymorphonuclear leucocytes and small round cells. Collections of both these types of cells were found scattered irregularly throughout the necrotic lesions. Nerve cells in the lesion of the occipital cortex showed all degrees of degeneration.

Dog 8 18 kg. A total dose of 630 mg. was given in twenty-one injections making an average daily dose of 1.49 mg. per kg. of body weight. Immediately after the first injection the animal whined and appeared restless. After this there were no apparent ill effects apart from rather marked swelling of the eyelids which came on a few minutes after some of the injections and lasted for several hours. The animal appeared healthy when it was killed 13 days after the course finished. No significant lesion of any kind was found postmortem either on naked-eye or histological examination.

Dog 9 4.5 kg. A total dose of 15 $\frac{1}{2}$  mg. was given in twenty-one injections making an average daily dose of 1.69 mg. per kg. of body weight. The animal tolerated the drug fairly well until the last 4 days, when it became inactive, had frequent shivering spells and appeared ill. It was killed 2 days after completion of the course. Postmortem examination revealed no significant lesions, either by naked-eye or histologically.

Dog 10 8.6 kg. A total dose of 315 mg. was given in twenty-one injections making an average daily dose of 1.74 mg. per kg. of body weight. There were no ill effects until on the 8th and 14th days when swelling of the eyelids and face occurred after both these injections. Immediately after the nineteenth injection the animal became unconscious. The head was retracted and the legs were spastic. It recovered consciousness in about 5 minutes, but until it was killed on the day after the twenty-first injection it remained weak and unable to stand, occasionally howling loudly as though it were in pain. Postmortem examination revealed greyish red regions of softening and haemorrhage in the right frontal cortex, both optic thalami and throughout the cerebellum, especially in that part forming the roof of the fourth ventricle. Microscopically these lesions consisted of degeneration, haemorrhage and infiltration with numerous polymorphonuclear leucocytes. Many of the small blood vessels had thickened walls which were infiltrated with polymor

2



4



5

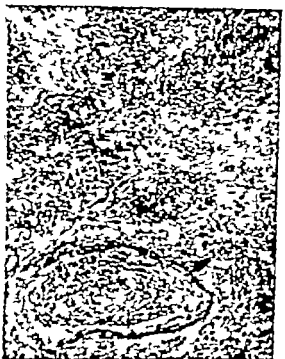


FIG. 2—Dog 3 on the 3rd day. Proximal part of the medulla oblongata ( $\times 700$ ). The wall of the blood vessel heavily infiltrated with polymorphs and small round cells, and there is extravasation of erythrocytes round the vessel. A degenerate nerve cell shows karyolysis.

FIG. 3—Dog 4 on the 8th day. Optic thalamus ( $\times 400$ ). There is myelin degeneration and infiltration with polymorphonuclear leucocytes. The blood vessel shows early thrombosis.

FIG. 4—Dog 6 on the 4th day. Caudate nucleus ( $\times 400$ ). Section through edge of lesion. Myelin degeneration, slight haemorrhage and infiltration with numerous large pigment-containing cells. Perivascular cuffing with small round cells.

FIG. 5—Dog 10 on the 2nd day. Optic thalamus ( $\times 700$ ). A thrombosed blood vessel. Walls thickened and infiltrated with polymorphonuclear leucocytes, small round cells, and erythrocytes. Extravasation of erythrocytes into surrounding brain tissue.



phomonuclear leucocytes and small round cells. Some of these vessels contained thrombi. Nerve cells showed all degrees of degeneration depending upon their proximity to the lesion (Fig 5)

#### SUMMARY OF RESULTS

The results of these experiments show that five of the ten dogs had clinical manifestations of central nervous system damage and that in all of these five animals macroscopic as well as microscopic lesions were found postmortem. The one animal which had recovered from early neurological signs and was killed 3 weeks after completion of the course, showed a healing lesion in each caudate nucleus. The five other animals had no clinical signs that could be definitely attributed to the central nervous system: three of these showed nothing abnormal in the brain, but in the remaining two there were microscopic lesions in the brain stem, cerebral cortex and spinal cord.

The clinical manifestations were very characteristic, the chief feature being extreme and generalized apasticity: the condition resembled that of decerebrate rigidity.

The liver was essentially normal in all animals except one in which there were small scattered foci of central lobular necrosis and a moderate degree of fatty change. The kidneys were normal except in five of the animals where there were slight fatty degenerations in the loops of Henle as shown by Sharlach R preparations.

There was no apparent relationship between the degree of neurological damage and the amount of drug which was given. Thus in Dog 3 1.46 mg per kg was given daily for twenty one injections and produced only minimal lesions, while in Dog 7 four injections of 1.06 mg per kg produced very extensive damage.

#### COMMENT

The pathogenesis of these selective cerebral lesions is not easy to explain. The small vascular lesions found in Dogs 2 and 3 are probably very early and minimal examples of the gross lesions seen in some of the other animals. This vascular lesion may be the primary condition which is followed by intravascular thrombosis, myelin degeneration and infiltration with erythrocytes and leucocytes. The sudden onset of ataxia or unconsciousness which was seen in some of the animals immediately after the injection suggests that vascular spasm and local anoxia may be important factors.

The toxicity of the drug is known to vary considerably in different animals. LOURIE and YORKE (1939) found that mice and rabbits survived relatively large single doses, but that puppies were more susceptible although they were able to tolerate doses of 5 mg per kg body weight. The cerebral lesions which we have described may represent only a special tissue susceptibility of dogs. On the other hand there is clinical evidence to indicate that damage to the central nervous system occurs in patients receiving treatment with 4-4-diamidino stilbene. HIRK and SATI (1940) reported two cases in which the closely related 4-4-diamidino diphenoxy pentane caused alarming epileptiform seizures and



The cephalin cholesterol flocculation test seems to fulfil both requirements. It is the purpose of this communication to present the behaviour of this test in five cases of infective hepatitis detected in the pre icteric phase and in four cases developing in contacts without an icteric phase.

### METHODS

Due to the lack of facilities, the deliberate production of infective hepatitis in volunteers could not be resorted to. I had to depend, therefore, on the early detection of cases with a natural infection. This was done by routine determinations of the cephalin flocculation test on sera of patients admitted to the wards of the American University Hospitals at Beirut, with the complaint of general "run down" health. Two cases were detected by this way. The other procedure followed was to perform the test on the sera of contacts in a small outbreak of infectious jaundice in Anfi, a village in Northern Lebanon. In this way three cases in the pre-icteric phase and four cases of the non-icteric types were detected. The details of the preparation of the testing emulsion, the reading and recording of results, are to be found in the original work of HANGER (1938).

### RESULTS

1. Group I—All of the five cases detected in the pre-icteric stage of infective hepatitis were strongly positive in the cephalin test (being ++ in two cases and +++ in three cases) at periods varying from 5 to 11 days before the development of clinical jaundice. Coincident with or shortly after the development of icterus a climax of ++++ was reached. This was maintained for a period of 10 to 15 days dependent on the severity of the conditions, to be followed by a decline in the curve to negative coincident with clinical recovery. A brief account of Case I is given here to illustrate the point —

J. P. (Hospital Case No. 41142). A 38-year-old English captain who came in for a "check up" examination on 10th January 1943 during a short leave. He had been in the Near East for 6 months prior to admission. For the last month he complained of a decrease in appetite with a feeling of fatigue and being "run down." On admission diagnosis of amoebic colitis and hepatitis was made. His liver was slightly enlarged and tender reaching to 1 cm. below the costal margin. Stool examination was negative for amoebae, but the cephalin test gave a +++ reaction. It was pointed out to the treating physician that the case might be one of infective hepatitis in its pre icteric phase. On 15th January i.e., 5 days later clinical jaundice was apparent. This was coincident with a rise in the cephalin test to ++++ and of the icteric index to 40. On 28th January the cephalin test became +++ and the icteric index 25. On 4th February patient was discharged from the hospital as improved. His icteric index dropped to 8 and the cephalin test to ++.

2. Group II.—This refers to the four cases presenting the picture of infective hepatitis with fever and a tender and enlarged liver developing in individuals in contact with cases of infective hepatitis but without clinical icterus. In all four cases the cephalin flocculation test was ++++

TABLE.

ESTABLISHED CASES OF INFECTIOUS JAUNDICE DETECTED IN THE PRE ICTERIC PHASE BY THE CEPHALIN TEST

No.	Initials	Age	Sex	Complaint	Enlarge- ment of liver	Date	Cephalin test	Notes
1	J P	38	M.	"Run down" feeling and loss of appetite	1 cm.	10.1.43	+++	No jaundice. Came for check up during his leave from Army. Admission diagnosis out.
						15.1.43	++++	Jaundice apparent. Icteric Index 40.
						28.1.43	+++	Jaundice less marked. Icteric Index 23.
						4.2.43	±	Pt. discharged. Icteric Index 8.
2	N S	21	F	Nausea with mild abdominal pain	2 cm.	14.10.44	+++	No jaundice. Admitted for study.
						20.10.44	++++	Jaundice apparent. Icteric Index 43.
						27.10.44	++++	Jaundice intense. Icteric Index 75.
						7.11.44	+++	Jaundice less marked. Icteric Index 49.
						12.11.44	++	Jaundice less marked. Icteric Index —.
						18.11.44	±	Pt. discharged. Icteric Index 11.
3	B. M.	26	F	Loss of energy	±	12.8.44	++	No jaundice. Was having a jaundiced appearance at home.
						23.8.44	+++	Developed clinical jaundice.
4	N S	19	M.	Irritability	Neg	12.8.44	++	No jaundice. No member of family had jaundice.
						22.8.44	++++	Developed clinical jaundice.
5	H. J	29	M.	"Run down" feeling	1 cm.	12.8.44	+++	No jaundice. Consistent with jaundice. Consistent.
						21.8.44	++++	Developed clinical jaundice.

3. Group III.—This refers to three cases of spirochaetal jaundice. Of these one was a case of Weil's disease due to *Leptospira icterohaemorrhagiae*. The cephalin test was found to be +++ in this case. The other two were cases of relapsing fever due to *Spirochaeta recurrentis*. The cephalin test was ++ in one case and +++ in the other.

#### DISCUSSION

In an endeavour to solve the problem of serum jaundice, FINDLAY MACCALLUM and MURGATROYD (1939), as well as BEZSON (1943), have suggested the rejection of potential blood donors who give a history of recent non-surgical jaundice. Since sera from cases in the pre-icteric phase were found to be infective to volunteers by OLIPHANT and his associates (1943) and since many cases of infective hepatitis do not develop an icteric phase, such a suggestion does not provide for enough protection.

More recently MACCALLUM and BAUER (1944) kept their sera for a month before use, watching their donors in the meanwhile for the development of any infective disease. This procedure cannot be applied where transfusions are needed under emergency conditions.

LOUITT and MAXWELL (1945) recommend that sera for prophylactic use should be individual sera. They refer to the finding by ANDREWS (1944) that dilution of a virus may increase its virulence, that the virus survives Seitz filter and drying process and that it may even be stabilized thereby. These authors admit, however, that the amount obtained from one donor is inadequate as a therapeutic unit for transfusion, and that it has the disadvantage of having unwanted iso-agglutinins. It is evident that the crux of the whole problem is that of detecting icterogenic sera before bleeding and consequent pooling. The present findings indicate that this is best accomplished by means of the cephalin test. It is suggested that the test be carried out, along with the Wassermann reaction, on all prospective blood donors, and that negative reactors to either test be included in the certified donors' list only. In case of donors who have been in malarial regions, the intradermal test in malaria described by the author (MAKARI, 1946) should supplement this test to exclude cases of latent malaria where the infection may be transmissible and where the cephalin test is negative.

#### SUMMARY

1. Five cases of infective hepatitis were detected in the pre-icteric stage by use of the cephalin-cholesterol flocculation test from 5 to 11 days before the development of clinical icterus.

2. Four cases of infective hepatitis without icterus showed also a strongly positive reaction in the cephalin test.

3. The cephalin test was also found to be positive in a proved case of Weil's disease and in two proved cases of relapsing fever.

4 Due to the simplicity and reliability of the cephalin test, it is suggested that it be used by private practitioners in their clinics to help in the detection of pre icteric and non icteric cases of infectious hepatitis

5 It is also suggested that the cephalin test be employed by all transfusion units on all prospective blood or plasma donors to exclude infective hepatitis as well as active malaria. Such a procedure would eliminate those cases of homologous serum jaundice where the icterogenic agent is the virus of infective hepatitis

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## CORRESPONDENCE

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### *PULLEX IRRITANS*

To the Editor TRANSACTIONS of the Royal Society of Tropical Medicine and Hygiene

SIR,

MAC ARTHUR (1948 *Trans R Soc trop Med Hyg* 39, 343) has drawn attention to the fact that the human flea *Pulex irritans* which has been shown to be capable of transmitting plague can adapt itself to a host other than man. From the epidemiological point of view this is rather important, especially when the host selected is the house rat. The following records from the Flea Survey of the Union of South Africa are of some interest in this connection.

These records do not prove that the human flea has become completely adapted to the hosts mentioned. Some of the records may be due to straggling, but they give the impression that *P. irritans* is not so restricted in its choice of host as is generally believed.

The figures in brackets relate to the number of occasions on which *P. irritans* was found on the host mentioned. *Rattus rattus* (31) man and bedding (18) plague house or on human case of plague (7) *Mus musculus* plague infected (1) *Mus musculus* uninfected (3) domestic dog (4) domestic pig (1) domestic calf (1) *Rhabdomys pumilio* (2) *Myotomys* sp (1) *Geosciurus leucurus* (2) *Suricata suricatta* (1) *Otomys irroratus* (1)

B. DE MEILLON

South African Institute for Medical Research.

D. H. S. DAVIS,

Union Health Department.

## DEATH OF TWO PAST PRESIDENTS

S P JAMES AND J W W STEPHENS

It is with much regret that we have to report the death of two of the Society's Past Presidents both of them distinguished in the field of malaria.

Lieut.-Colonel S P James F.R.S., who died on 17th April, was a member of the Indian Medical Service and later Malaria Officer to the Ministry of Health and Director of the Malaria Laboratories at Horton Mental Hospital. In India he contributed much to our knowledge of the mosquitoes of that country with special reference to malaria and filariasis. While at the Ministry of Health he organized from the laboratory at Horton the malaria treatment of mental patients throughout the country and was thereby enabled to make valuable contributions to our knowledge of the action of quinine and the behaviour of malarial parasites in the human body particularly from the point of view of relapses and their underlying causes. This work led him to the study of the malarial parasite of fowls and the discovery with TATE at Cambridge of the extraordinary development in cells of the reticulo-endothelial system to which he gave the very descriptive if somewhat ponderous title *exoerythrocytic schizogony* which will ever be associated with his name. To the day of his death his active mind was occupied with the possibilities of demonstrating a similar cycle in human malaria, for he realized that this would solve many of the problems which confronted him at Horton and explain much of what is still obscure in the course and treatment of this world wide disease.

The second Past President whose death we have to announce is Professor J W W STEPHENS F.R.S. who died on 17th May 1946. He was a member of the Royal Society's Commission on Malaria in Africa and India, 1898-1902 with another of our Past Presidents, SIR RICKARD CHRISTOPHERS. These two added much to our knowledge of mosquito malaria relationships in the early days following SIR RONALD ROSS's discovery of mosquito transmission. Later Professor STEPHENS occupied the chair of Tropical Medicine at the Liverpool School of Tropical Medicine, where he brought his critical judgment to bear on problem of malaria and blackwater fever and for many years imparted his knowledge to a long line of students who passed through the School.



# ANNOUNCEMENTS

## THE NEXT MEETING OF THE SOCIETY

The Opening Meeting of the 40th Session will be held at Manson House on Thursday 17th October 1946 at 8 p.m.

## MOVEMENTS OF FELLOWS

The following Fellows from abroad have notified the Secretaries that they are *temporarily* in the British Isles. Letters addressed to any of these care of the Royal Society of Tropical Medicine and Hygiene, Manson House 26 Portland Place, London, W 1 can usually be forwarded to the home address.

To ensure the accuracy of this list Fellows named below are particularly requested to advise the Secretaries when they return to their stations abroad

ABELHEIM E. S Africa.	LEVER R. J A. W., Fiji.
ANDERSON Capt E. S., M.E.F	LE ROUX, P L. Northern Rhodesia
ANDREW Col R. R., Australia.	MCArTHUR, J N British North Borneo.
BALL, J D India.	MACKAY S J C. Egypt.
BAXTER, G R., Fiji	MACKAY Wing Comdr IAN
BEATTIE, MARY V F Trinidad	McKENZIE, ALAN Tanganyika Territory
BERRY W T C., Nyassaland.	MACKIE, T T U.S.A.
BIGGER W K. Palestine	MACKIE, J W U.S.A.
BLAKEMORE, W L. Malaya.	McLEAN N., Kenya.
BOWKER, C M. Portugal	McPHERSON A Gold Coast
BRAINE, G I H Malaya.	McPHERSON D ROSS Malaya.
BRANCH A G M Nigeria	MAKARI J Syria.
BROWN Rev D M., Northern Rhodesia.	MALAMOS, Prof B., Greece.
BRYAN T B L., Uganda.	MANNON J K. India
BUSBY J C., Sierra Leone.	NICHIE, CHARLES Sierra Leone.
CARNICHAEL, JAMES, Uganda	RAMSAY G C., India.
CHWATT, Major L. J., India.	RAO, T R. India.
CLARK, R. H. P. Calcutta.	REED HOWARD Tanganyika Territory
COCHRANE, R. G., India.	ROBERTS, J I., Kenya.
COLLINS, J., Nyassaland.	ROBINSON C. Gold Coast
COPPELAND F J India.	ROSS, C. C., Malaya.
DALY E. J. Gold Coast.	ROUTLEY P E. F., Singapore
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EARLE, K. V Ecuador	SCOTT, A. Malaya.
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HARGREAVES H E. Persia.	TIDMAN D A. S.E.A.A.F
HOLLINS, C. Nigeria.	TREGARTHEN J C. T., British North Borneo
HUNTER, W S Nigeria.	TWINING HELEN M. Mauritius.
INNES, J R. India.	WATSON W HALL, Malaya.
JACQUES, J V Malaya.	WILSON J V., Egypt
KIRK, R. Sudan.	WILSON, T., Malaya.
KNOTT H T Belgian Congo.	WIDEMAN R H., Kenya.
LAMBERT W A., Tanganyika.	WORTH H. NORMAN S Rhodesia.
LANDOR, J V., Malaya.	WRIGHT S C., Gold Coast.
LEECH, R. B. Uganda.	ZARBA NEUMANN C., Malta.





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